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The Mechanism in the Development of Pulmonary Edema.

LEO LOEB.

From the Department of Pathology, Washington University School of Medicine.

In a paper recently published, Johnson[1] gives an account of 2 sets of experiments in which it was possible to prevent the development of pulmonary edema which otherwise would have occurred following the injection of adrenalin and caffein in rabbits. The first method consisted in the opening of the chest wall. In experiments independently carried out by both the writer in association with Dr. M. S. Fleisher, and by Dr. Johnson, the occurrence of pulmonary edema was prevented by this procedure. The second method consisted in the insufflation of air into the lung. Dr. Johnson attributes the prevention of pulmonary edema in these cases to increased pressure exerted on the walls of the capillaries of the lung acting in the direction from the alveoli towards the pleural surface. This increased pressure on the vessels tends to compress them and to counteract the increased pressure which obtains in the lumen of the vessel which latter is caused by the obstacle to the outflow of blood from the pulmonary veins produced by the spasm of the left ventricle and contraction of the peripheral arterioles. We believe that these considerations are of significance in the analysis of pulmonary edema and that the pressure exerted on the pulmonary vessels is an important factor in edema of the lung, but we suggest a modification in the definition of the manner through which the outside pressure acting on the pulmonary vessels influences the development of edema.

We believe that in the experiments of Dr. Johnson as well as in other cases of pulmonary edema the negative pressure which develops in the pleural cavity as well as in the alveoli of the lung during the phase of inspiration tends to suck out the fluid from the vessels concomitantly with the sucking of the air from the outside into the alveoli. The more intense the inspiratory movements are, the greater the tendency to withdraw fluid from the blood, provided other conditions make such a transudation possible. There are various factors which fulfill the latter condition, namely, (1) the overdistension of the pulmonary vessels, probably associated with increased blood pressure following injection of a large amount of adrenalin, or of a small dose of adrenalin combined with caffein. (2) An increased permeability of the pulmonary vessels, perhaps associated with inflammatory congestion, as the result of the inhalation of irritating gases. (3) In case the entrance of air into the alveoli of the lung is inhibited through occlusion of the bronchi or trachea, the occurrence of pulmonary edema is due to increased inspiratory efforts and to increase in the negative pressure in the alveoli, and subsequent increase in the sucking force acting on the capillary blood in the walls of the alveoli and aspirating the fluid into the alveolar spaces.

All those measures which diminish the negative pressure during inspiration and the consequent sucking action should prevent pulmonary edema or at least decrease its intensity. Both the methods employed by Dr. Johnson, namely, admitting air from the outside through an opening in the chestwall as well as insufflation of air through the trachea, may thus be expected to diminish the negative pressure in the alveoli during inspiration and diminish pulmonary edema. We believe, therefore, that it is not only the dilatation of the vessels and the subsequent increase in permeability of the vessels which have to be considered in this connection, but also a direct aspirating effect, developing as the result of negative pressure, on the fluid in the dilated vessels, and this sucking effect should be expected to take place during inspiration.

In the case of pleural effusions, Graham[2] has suggested that the negative pressure obtaining within the thoracic cavity during inspiration might force out the fluid into the pleural cavity. However, in this case, experiments *in vitro* by Graham showed an actual squeezing out of the fluid to occur during the expiratory phase. The conditions determining the production of pleural effusion under the conditions obtaining *in vitro* and the formation of pulmonary edema in the living animal are not identical. Our conclusions are in accord with the findings of Auer and Gates,[3] who observed that in

rabbits in which the vagus has been cut, insufflation of air into the bronchi prevented edema of the lung, and who attributed this effect to the diminution in negative pressure during expiration. These authors believe that negative pressure is an important factor in the development of pulmonary edema. We conclude that also the opening of the chest prevents or diminishes edema of the lung by this same mechanism.

[1] Johnson, Scott, PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 181.
[2] Graham, E. A., *J. Am. Med. Assn.*, 1921, lxxvi, 784.
[3] Auer, John, and Gates, F. L., *J. Exp. Med.*, 1917, xxvi, 201.

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The Specific Action of Salts in Preparation of Urease from Amoebocyte Tissue of Limulus.

LEO LOEB AND I. LORBERBLATT.

From the Department of Pathology, Washington University School of Medicine.

In previous investigations Loeb and Bodansky[1] found that different salts have a specific action in the extraction of urease from amoebocyte tissue of Limulus, in accordance with the character of the kations. The salts of alkaline earths are by far the most favorable; the salts of alkali metals are very unfavorable, and the salts of Mg and in decreasing order Mn are intermediate in effectiveness. While extracts prepared with salts of heavy metals are apparently of similar strength to those prepared with salts of alkali metals, the addition of the salts of heavy metals to active extracts is very much more injurious than the addition of salts of alkali metals.

We recently analyzed the effect of mixtures of salts in the preparation of the urease, and the effect of addition of salts after the extraction with certain salts had been completed. If mixtures of salts are used in the preparation of extracts, the activity of the extracts thus prepared is approximately intermediate between the activity that is characteristic of each component salt; this applies to various combinations of NaCl, $MgCl_2$ and $CaCl_2$. If on the other hand, the extraction is first completed and then another salt is added, the results vary in accordance with the salt used for extraction. If we extract with NaCl and add a more favorable salt to the extract the addition of the latter salt is ineffective. On the other hand, if the extract has been prepared with $MgCl_2$, the subsequent addition of $CaCl_2$ leads to a condition in which the activity coefficient is inter-

mediate between the activity coefficient of $MgCl_2$ extract and the activity coefficient of $CaCl_2$ extract.

While extraction with Limulus serum in which the preformed urease has been previously inactivated through heating to 80° for 30 minutes, is more favorable than extraction with 0.5 m. NaCl solution, only a slight improvement in the activity of this extract can be produced through addition of $CaCl_2$ at the beginning of the extraction, and the effect is injurious if $CaCl_2$ is added to the extract. On the other hand, if we use unheated Limulus serum as extractive, the extract is much more potent than that prepared with heated serum, and in this case the activity coefficient of the preparation is increased through addition of $CaCl_2$ previous to extraction as well as after completed extraction. If we substitute for Limulus (or Lobster) serum indifferent colloids, like gum arabic, no effect is produced. Addition of salts to the preformed urease, as present in fresh Limulus serum, is injurious.

The lowering of the pH which we observe after addition of $CaCl_2$ to the extractive or to the extract depends probably largely on the formation of $CaCO_3$ as the result of the interaction between $CaCl_2$ and $(NH_4)_2CO_3$; in addition there takes place in all probability an interaction between a buffer substance in the $CaCl_2$ extract and the alkali which is produced in the course of the urease action.

Tentatively we may assume that urease or a substance associated with the enzyme, or a substance which exerts a strong influence on the activity of the enzyme, combines with various kations and that the activity coefficients of the urease in these combinations differ greatly. While the Mg combination is not so potent as the combinations with Ca, Ba or Sr, the former preserves the enzyme in such a condition that Ca can take the place of Mg in such a combination. Therefore in a mixture of Ca and Mg salts, both Mg and Ca combinations seem to form side by side and the activity coefficients are, therefore, intermediate. On the other hand, the combinations with alkali metals are apparently of such a nature that the enzyme is changed irreversibly and that subsequently no effective combinations with Mg or Ca can be produced. It is, therefore, necessary to add the salt of the alkali earth to the salt of the alkali metal in the beginning of extraction, before the alkali metal has had a chance to enter into a combination with the enzyme or with associated substances, if we wish to increase the activity coefficient above that of the NaCl extract.

We may conclude that the specific effect of the salts in these experiments does not depend upon their specificity in the process of extraction as such, but upon their interaction with the enzyme or

with a specific associated substance which takes place as soon as the latter has been made accessible to the salt.

Furthermore, it seems that we can distinguish at least 3 different effects of salts in their interaction with the urease: (1) Specific combinations of kations with the enzyme or with associated substances. In these combinations alkali earths are by far the most favorable. (2) Injurious salt effects of a non-specific character. Salts of heavy metals are more injurious than salts of alkali earths and the latter are more injurious than salts of alkali metals. (3) In addition osmotic effects of salts play in all probability a certain rôle. Increased osmotic pressure of salts or of non-electrolyses, at least within the range we have examined so far, acts favorably on the enzyme. Furthermore, we have found specific actions of proteins which may modify the effect of salts on urease.

[1] Loeb, Leo, and Bodansky, O., *J. Biol. Chem.*, 1927, lxxii, 415.

3839

Sex Characteristics in Monkeys.

EDGAR ALLEN.

*From the Department of Anatomy, University of Missouri.**

Female monkeys during sexually mature life show the sex characteristics of reddening and swelling of the skin about the external genital organs, on the buttocks and medial surfaces of the thighs. These phenomena disappear after double ovariectomy and can again be induced in spayed animals by injections of ovarian and placental extracts.[1] Three male monkeys in our colony had never shown this cutaneous reddening. (It has, however, been reported that male monkeys may show this reddening during the breeding season.) During the late summer and fall the 'sexual skin' of a large 7-year-old male began to redden. During November and December the regions affected covered an area 15x13 cm. in extent. The reddening reached a moderate intensity but never approached the maximum 'sexual skin' color of the mature female. From our experiments with ovarian hormone in inducing this condition in spayed animals, it seemed logical to infer that this condition in the male might be due to the male sex hormone.

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Needels[2] had previously reported a series of blood tests in our monkeys by a modified Manoilov technique, a test supposed to distinguish male from female blood. To our great surprise the blood of this fully grown, finely developed, 6-year-old male had consistently given a 'female' reaction. Two younger males, one of which was immature, returned typical 'male' tests. This test seemed essentially a reduction reaction, the male blood usually causing a greater reduction of the reagents than the female blood. But Needel's results indicated fluctuation especially in females when tests were repeated upon the same individuals. Consequently a graded series of color standards were prepared for comparison. The tests ranged from no decolorization ('female') to complete decolorization ('male').

Several conclusions were drawn: (1) There was a distinct variation in individual female monkeys during the menstrual cycle. At or near menstruation the tests showed less decolorization, while during the intermenstrum greater decolorization (an approach toward the 'male' reaction) occurred. The intermenstrum is the time when follicles are largest and secondary sex phenomena most marked—the time when the ovaries are supposedly most actively secreting hormone.[1, 3] Consequently, if this reaction were specific for sex, the opposite variation, toward the other ('female') end of the scale, would be expected. (2) After ovariectomy less decolorization of reagents occurred. (3) When ovarian and placental extracts in oil were injected into the ovariectomized animals there was a greater decolorization—a shift toward the 'male' end of the scale.

Needels agreed with Riddle and Reinhart[4] in the opinion that this test, instead of being specific for sex, was really dependent upon metabolic level. In a majority of cases, however, the Manoilov reaction did place the individual as to sex.

Since the skin reactions under consideration are similar, the blood reaction (Manoilov) of the normal female when ova are ripening approaches the 'male' reaction and the particular male described had consistently given a 'female' reaction, one might infer a similarity in male and female sex hormones. Such a similarity has been reported by Fellner,[5] Robinson and Zondek,[6] and Laquer.[7] They find that extracts of testis injected into female test animals may give results somewhat similar to ovarian extracts. Doisy, Ralls, Allen and Johnston[8] reported negative results in attempts to induce oestrus in spayed rats from injections of lipoid extract of beef testicle.

The success of Smith and Engle[9] and of Zondek and Aschheim[10] in the use of 'implantation' of fresh tissues for the demonstration of

hormone effects and the limited amount of testicular tissue available suggested the use of fresh material. Consequently, on December 13th the left testis was removed from this monkey. It measured 49.5x36x34 mm. and weighed (without the epididymis) 31.38 gm. The weight of the epididymis alone was 4.42 gm. Comparison of the weight of this testis with the average cited for the human testis (10.5 to 14 gm.[11]) shows it to be more than twice the average for man, in spite of the fact that this full grown monkey weighed less than 30 pounds. Sperm from the epididymis and proximal portion of the *vas deferens* were extremely motile. The testis and epididymis were cut up separately, ground up in a small amount of Ringer's solution and injected subcutaneously into a series of 10 ovariectomized rats. Four rats received five 1 cc. doses spaced over 26½ hours, 3 rats received 4 injections, and 3 rats received 3 injections during shorter periods. The dosage of testis substance received by the rats ranged from 1/12 to 1/20 of the total testis. The dosage of epididymis substance ranged from 1/4 to 1/7 of the total. The Ringer suspension was kept on ice in a rubber stoppered sterile bottle during the interval to minimize deterioration. In none of the 10 rats injected was there the slightest indication of even a partial oestrous reaction as determined by the vaginal smear method.

These results, therefore, offer no positive evidence for a similarity of action of testicular hormone to that of ovarian hormone in producing substitution effects in spayed females. They do not, however, rule out the possibility that highly concentrated extracts might do so.

From Needels' results with the Manoilov test it would seem that both male and female hormones act in a similar way (but in different degrees) upon the reduction factor of the blood. Surely the 'sexual skin' characteristics of the large male described were very similar to such conditions in the female.

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[1] Allen, E., Carnegie Inst., Washington, 1927, Pub. No. 380, 1.

[2] Needels, L. J., *Anat. Rec.*, 1927, xxxii, 238.

[3] Corner, G. W., Carnegie Inst., Washington, 1923, Pub. No. 332, 73.

[4] Riddle, O., and Reinhart, W. H., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 359.

[5] Fellner, O. O., *Pflüger's Arch.*, 1921, clxxxix, 199.

[6] Robinson, M. R., and Zondek, B., *Am. J. Obst. and Gyn.*, 1924, vii, 83.

[7] Laquer, E., *Klin. Wochenschr.*, 1927, vi, 390.

[8] Doisy, E. A., Ralls, J. O., Allen, E., and Johnston, C. G., *J. Biol. Chem.*, 1924, lxi, 711.

[9] Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927, xl, 159.

[10] Zondek, B., and Aschheim, S., *Klin. Wochenschr.*, 1927, vi, 248.

[11] Gray's Anatomy.

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Selective Distribution of Portal Blood in the Liver. An Experimental Study.

GLOVER H. COPHER AND BRUCE M. DICK.* (Introduced by E. A. Graham.)

From the Department of Surgery, Washington University School of Medicine, and Barnes Hospital, St. Louis.

A few experimental and clinical studies have suggested the possible occurrence of selective distribution of the portal blood in the liver, and the presence of independent currents of blood in the portal vein. The existence of such segregated streams of blood in the portal vein and their subsequent distribution to definite parts of the liver should be of considerable practical significance in the physiology and pathology of the liver. Serege,[1] Glenard[2] and others found that India ink was deposited in the left lobe of the liver after injection of the splenic vein and into the right lobe of the dog's liver after injection into the mesenteric vein. They concluded a dual portal current existed which determined the selective hepatic distribution. Bauer[3] and other workers were unable to confirm these observations. Bartlett, Corper and Long,[4] after studying the distribution of fat emboli in the liver after injection of olive oil in various portal tributaries, concluded that the dog has a dual portal current and that there is a selective distribution of portal blood in the liver.

We made a study of the blood currents in the portal vein itself and observed their subsequent distribution in the lobes of the liver. Trypan blue injected into the veins of the stomach and spleen was conveyed by the blood to the left half of the liver almost entirely. Dye injected into the veins of the upper part of the duodenum, the head of the pancreas and jejunum was carried to the 2 right lateral lobes almost exclusively. When the veins of the colon were injected, the dye was distributed to all parts of the liver, but more particularly to the large lobe of the left side.

An explanation for the selective distribution of the portal blood in the liver is offered by the fact that separate currents of blood in the main portal channel can be demonstrated by transillumination of the vessel after injection of dye into the main tributaries of the portal vein.

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*Fellow in Surgery, Rockefeller Foundation.

[1] Sérégé, H., *J. de Méd. de Bordeaux*, 1901, xxxi, 271, 291, 312.

[2] Glenard, F., *Bull. et Mém. Soc. Méd. de Hop. de Par.*, 1901, 3, 18, 386.

[3] Bauer, A., and Brissant, E., *J. de l'anat. et de la Physiol.*, 1909, xlv, 1.

[4] Bartlett, Corper and Long, *Am. J. Physiol.*, 1914, 35, 36.

3841

The Uretero-Vesical Valve and Experimental Production of Hydroureter Without Obstruction.

CHARLES M. GRUBER.

From the Department of Pharmacology, Washington University School of Medicine.

Many observations, both experimental and clinical, have been made to support or disprove the theory that the urine flows back into the ureter from the bladder. All early writers and many of the present time maintain the "vesical-ureteral sphincter" competent to withstand any pressure. Many men have alluded to the valve mechanism as preventing reflux yet most refer again to it as a "sphincter". The oblique passage of the ureter through the bladder wall has been regarded as a sphincter in preventing reflux of urine.

Without experimental evidence, Sampson[1] in 1903, was the first to attribute to the intra-vesical ureter the function of a valve. He placed little value on his findings since his oblique transplants of the ureter without the valve also showed no reflux. Delbet[2] denies valve action to the intravesical ureter. Satani[3] believed that normally there is no reflux into the ureter although he found no special structures of a nature of a sphincter in the bladder. After destroying all the muscles of the bladder wall which cover the intra-parietal part of the ureter he found the ureter still fairly tight against reflux under intravesical pressure.

Excised bladders with ureters attached were used in this work in most experiments. These were obtained from human, monkey, baboon, dog, cat, rabbit and pig. Cannulae were tied in the ureters and these were attached to mercury manometers. A large brass tube, with a window at one end, into which ran inlet and outlet tubes, was fixed in the dome of the bladder, the urethra being tied off. A pressure bottle containing either normal salt solution or Ringer's solution was fastened with a long rubber tube to the inlet tube and a mercury manometer to the outlet tubes. In all bladders tested, increasing the intra-vesical pressure by raising the pressure bottle did not increase in intra-ureteral pressure even though the pressure was sufficient to rupture the bladder.

In some bladders, one intra-vesical ureter was excised completely, leaving the oblique passage of the ureter through the bladder wall intact. When the intra-vesical pressure was increased in these the ureter from which the intra-vesical portion was excised showed the

same pressure as that within the bladder, while the normal ureter showed a zero pressure. Such bladders were kept in the ice-box as long as 20 days after excision and tested from time to time. In most of these the intact valve was found competent in dog and pig bladders during the entire period. In some, however, there was re-

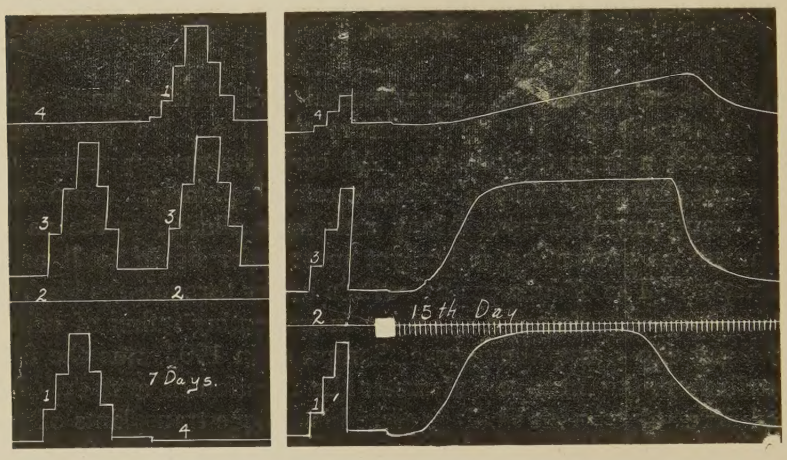

FIG. 1.

gurgitation by the so-called normal valve on the fifteenth day. Fig. 1 is such a record taken with a pig's bladder. In this figure, 1, is the changes in pressure within the ureter in mm. Hg. with the intra-vesical ureter cut; 2, horizontal line and time in 2 second intervals; 3, the change in intra-vesical pressure in mm. Hg., and 4, the change in intra-ureteral pressure with the intra-vesical ureter unaltered by operation. On the first as well as on the seventh day the intra-ureteral pressure in 1 follows closely the intra-vesical pressure, while that in 4 remains at zero. On the fifteenth day some reflux is observed also in 4. This, however, is much slower than in 1, in which the intra-vesical ureter is missing.

Unilateral enormously dilated ureters were obtained from 2 pigs. In both instances the valve to the hydro-ureter was patent. The intra-ureteral pressure without destruction of the valve became at all times the same as that in the bladder.

In dogs under ether anesthesia the intra-vesical ureter was excised on one side and the animals allowed to recover. After 5 months or longer the animals were killed and the ureters examined. In those animals in which the intra-vesical ureter was entirely removed and the ureter patent, hydro-ureters resulted.

We believe the intra-vesical ureter to serve as a true valve (ure-

tero-vesical valve) and complete excision of it leads to hydro-ureter and hydronephrosis. With an infected urine pyoureter and pyonephrosis are possible.

[1] Sampson, *Johns Hopkins Hospital Bull.*, 1903, xiv, 334.

[2] Delbet, Poirier and Charpy, *Traité D'Anatomie Humain*, 1907, v. i, 108.

[3] Satani, *J. Urol.*, 1919, iii, 247; *Am. J. Physiol.*, 1919, xlix, 474; *Ibid.*, 1919, 1920, l, 342.

3842

Anatomy of Fear.

ALFRED OGLE SHAKLEE.

From the Laboratory of Pharmacology, St. Louis University School of Medicine.

In the study of pigeons after the ablation of various amounts of brain tissue, it appeared that when a sufficiently large amount of brain was removed, there was a corresponding amount of fear removed; the larger the portion of brain removed, the greater the portion of fear removed. This fact, that fear can be removed in stages by removing increasing portions of brain, seems to indicate that there is no definitely localized center for fear. It seems that the fear network spreads throughout the brain, and even after the removal of all of the cerebral hemispheres, there still remains in the residual brain, a functional portion of the fear network.

I give some findings from 4 pigeons of a series now under study. In all pigeons there appeared to be a gradual recovery of ability to show fear signs.

Of the 4, No. 1, had most brain left, and while showing, in nearly 12 months study, very much less fear than the normal pigeon, it showed considerably more than any of the other 3. On the 40th day, after being fed artificially, he flew from my knee onto the table, showing marked semblance of fear. On the 58th day, after being put down from the platform balance, he showed evidence of fear and flew 15 cm. up onto the other platform, as if trying to escape. On the 168th day to avoid being taken up, he ran and flew with such vigor and persistence that it required quite a chase to catch him. On the 251st day, when we tried to take him up, he ran under the table and out on the other side, flew up into the air, circled around, passed over the revolving book case and down on the other side (height of flight 2±M.; length 4±M.). Flying increased as time went on.

Nos. 3 and 4 had less brain left than No. 1, but owing to the influence of the nutritive condition, which cannot be easily evaluated, it is difficult to decide from the evidence, whether No. 3 or No. 4 was capable of showing the more marked fear signs. The evidence collected thus far leaves in my mind the impression that No. 3 was the more capable. I cite, here, 2 sets of facts: On the 11th day No. 3, when grasped gently, struggled considerably to get out of my hands; while, in the case of No. 4, this did not happen until the 24th day, in spite of the fact that the test had been made every day after the operation. (No. 1 escaped from my grasp on the 9th day.) Simple pursuit caused No. 3 to run away with the semblance of fear and the utterance of fear sounds, on the 41st day; while repeated trials failed to produce this reaction in No. 4 in 77 days.

No. 2 had the least amount of functional brain left and showed the least evidence of capacity for fear signs. She showed some slight fear reactions, for example: The sound of snapping the fingers caused opening of eyes and slight turning of the head toward the sound; the rapid approach of the hands on the 6th day caused only a slight dodge, and the same on the 141st day. On the 130th day she jumped off the balance platform onto a nearby box with some semblance of fear. On the 139th and 141st days the presence of a cat had no effect, even when the cat moved and struck at her. On the 141st day there were escape movements of legs and wings when she was in my grasp. On the 142nd day, the sudden appearance of the cat, once caused a sudden slight start back with no semblance of fear. Other trials caused only a slight halt in her walk, or nothing at all. On the 145th day, she looked at the slowly approaching hand; once she arched her neck; once she took 1 or 2 steps back, with an expression that might have been taken for very slight semblance of fear, or could easily have been taken for curiosity. In nearly 5 months she showed only the barest semblance of fear, and those, on very few occasions. All attempts to scare her failed to produce any flying, any fear sound, any running or any marked semblance of fear, unless the jumping from the platform of the balance or the escape movements in the grasp may be so regarded.

The Residual Brain of No. 1 was lost after it had been measured, photographed, and sent to the technician for microscopic preparation. Gross inspection, measurement, and examination of the photographs gave the following impressions: Irregular basal portions of the cerebral hemispheres remained. All the parts below the cerebral hemispheres were intact. The residue of the cerebral hemispheres showed the olfactory lobes and bulbs intact; a marginal roll on the

left side extending from the olfactory lobes to the vermis and varying in thickness from 1.5 mm. in front to 0.5 mm. in the occipital region. On the right side there was less tissue left. There remained a rounded mass near the center, more on the left of the base than on the right, about 4 mm. long and 2 mm. broad, with its transverse axis about over the centers of the optic lobes.

The residual brain of No. 3 has already been described.[1]

No. 4 is still living. Judging from the appearances and technique at the operation and succeeding symptoms, it is thought safe to assume that all cerebral tissue and a small portion of brain stem tissue in the right dorsal region, were removed.

The residual brain of No. 2 showed a retention of approximately the same median cerebral tissue at No. 3 but a loss of the lateral cerebral nucleus and of central and dorsal portions of the anterior region of the brain stem retained by No. 3.

[1] Shaklee, A. O., PROC. SOC. EXP. BIOL. AND MED., 1927, XXV, 186.

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Duodenal and Gastric Ulcers in Dogs With Biliary Fistulas.

BENJAMIN N. BERG, ALEXANDER JOHNSTON AND JAS. W. JOBLING.

From the Department of Pathology and Surgery, College of Physicians and Surgeons, Columbia University.

Although numerous methods have been employed to produce peptic ulcers experimentally,[1] the frequent occurrence of ulcers of the duodenum in dogs with bile fistulas has not been described. Bickel,[2] Exalto[3] and Mann[4] found ulcers in the jejunum after diverting the duodenal, biliary and pancreatic secretions away from the stomach and performing a gastro-jejunosotomy. Mann also found ulcers in the duodenum by transplanting the pancreatic and bile ducts into the lower ileum. It was considered that an important factor in the development of these ulcers was the loss of the neutralizing effect of the alkaline secretions upon gastric acidity. According to Boldyreff,[5] the alkalinity of the pancreatic secretions was greater than that of all the others combined and was the chief element in the neutralization of the gastric juice.

In a series of dogs with bile fistulas, we noted at autopsy the prevalence of duodenal ulcers. The fistulas were made according to the method of choledochostomy described by Rous and McMaster[6] which permitted the collection of the total output of hepatic bile and deprived the animals completely of their biliary secretions. The dogs could not lick the fistulous openings, and accessory communications between the common duct and the intestine were ruled out at autopsy.

The series consisted of 9 dogs which lived for periods extending from 12 days to 46 days. There were 8 males and one female. The ages varied. The diet consisted of combinations of biscuit, bread,

meat, oatmeal, cod liver oil and bones, with water *ad lib.* Fat was removed as completely as possible from the food. Two dogs received liver, in addition to the other food. The appetites of the dogs were capricious and it was found necessary to vary the constituents of the diet frequently. All of the dogs lost weight, the average loss being approximately 25% of the original weight. The stools were clay colored and well formed.

In 7 dogs duodenal ulcers were found 13, 14, 16, 24, 26, 29 and 46 days respectively after the establishment of the bile fistulas; in one of these dogs (26 days) a pyloric ulcer was also present; in another (46 days) 2 gastric ulcers were found in addition to a duodenal ulcer. In 2 dogs which were examined 12 and 15 days respectively after operation, no ulcers were found.

The characteristic features of the duodenal ulcers were as follows: they were single and were situated usually on the ventral surface of the duodenum between the pyloric sphincter and the ampulla of Vater, never encroaching upon the latter, however. The acute ulcers were irregularly oval and were found in 3 dogs (13, 14 and 16 days). The subacute or chronic ulcers were round and occurred in 4 dogs (24, 26, 29 and 46 days). The size varied from 25 mm. x 15 mm. to 5 mm. x 4 mm. The ulcers had a punched-out appearance and penetrated the muscularis of the intestinal wall. In the acute type, the base of the ulcer was shaggy and friable. In the subacute and chronic types, the margins were indurated and the bases were firm and hard; these ulcers were easily palpated before the intestine was opened. The microscopic appearance of the ulcers corresponded to the gross findings. There were no evidences of vascular changes in the region of the lesions. No histological alterations were observed in the pancreas. In 2 dogs death was due to suppurative peritonitis following perforated duodenal ulcers.

The gastric ulcers were found on the lesser curvature and measured 17 mm. x 10 mm. and 17 mm. x 5 mm. respectively. They were of the chronic indurated type and the margins were undermined. One of the ulcers was perforated. The pyloric ulcer was a shallow lesion measuring 10 mm. x 5 mm.

Duodenal ulcers occurred in a high percentage of dogs with bile fistulas. Gastric ulcers were also encountered. The lesions were similar to those found in man. Although no definite analogy can be made between the experimental findings and human ulcers at the present time, the isolation of a single controllable factor, such as the bile, in the experimental production of ulcers may be of value in further investigations. A control series of 50 dogs were examined

for the spontaneous occurrence of duodenal or gastric ulcers, but none were found.

[1] Hauser, G., Henke-Lubarsch, *Handbuch der Speziellen Pathologischen Anatomie und Histologie, Berlin,* Julius Springer, 1926, iv, 339.

[2] Bickel, A., *Berl. Klin. Wchnschr.,* 1909, xlvi, 1201.

[3] Exalto, J., *Muench. Med. Wchnschr.,* 1911, lviii, 1144; 1911, lviii, 1792.

[4] Mann, F. C., and Williamson, C. S., *Ann. Surg.,* 1923, lxxvii, 409.

[5] Boldyreff, W. N., *Quar. J. Exp. Physiol.,* 1914-15, viii, 1.

[6] Rous, P., and McMaster, P. D., *J. Exp. Med.,* 1923, xxxvii, 11.

3844

Effect of Hardening and Fixation on Gram Reaction.

JOHN W. CHURCHMAN.

From the Laboratory of Experimental Therapeutics, Cornell University Medical School.

In a recent study of sections of the livers and spleens removed from mice, dead of experimentally produced anthrax, we were surprised to find that although the young culture used to induce the disease had been sharply gram-positive, the majority of the organisms with which the tissues teemed, were either completely or partially decolorized by gram. The experiment was repeated with *Saccharomyces cerevisiae,* a sharply gram-positive organism, more stable in this respect than *B. anthracis.* A heavy suspension of this organism was injected into the anterior abdominal vein of an etherized frog after the other vessels of the liver had been ligated. The liver thus impregnated with yeasts was immediately removed, and pieces of it were put into Zenker's fluid and into formalin. These were run through in the usual manner and cut in paraffine. The sections were stained by gram. (Burke's Modification). A large majority of the organisms were found to be completely or partially decolorized. This reversal of the gram reaction (similar to that recently described as following exposure of *B. anthracis* to acriviolet[1]) might be thought of as occasioned by the contact of the organisms with the animal body, or as resulting from exposure of the organisms to the various chemicals used in hardening and fixation.

An inquiry was therefore instituted as to the effect of these processes on gram reaction. On account of the danger of this kind of experiment if done with *B. anthracis, Saccharomyces cerevisiae*

was used. A heavy suspension of these yeast cells was made and put into two tubes. To one of these Zenker's fluid was added, to the other formalin. At the end of 24 hours, the specimens were centrifugated. The sediment was washed, re-centrifugated and re-washed 10 times during the course of 24 hours. The centrifugate was then run through the alcohols, aniline oil, xylol, and 3 changes of paraffine, just as if sections were to be cut. Smears were made from the last paraffine and were fixed by air drying. The paraffine was removed with xylol, the xylol by alcohol, and the alcohol with water. The smears were stained by Burke's Method. A few entirely gram-positive cells were seen, but the majority were completely decolorized, just as they had been in the sections; that is to say, the processes of hardening and fixation alone had reversed the gram reaction of most of the organisms.

An experiment of another kind was done to test this point. *B. anthracis* was injected into a mouse, which died in 20 hours. Immediately after death, smears were made from liver, spleen and heart's blood. These contained large numbers of *B. anthracis,* practically all of which were sharply gram-positive. Pieces of liver and spleen were then put into Zenker's fluid and run up through the alcohols, aniline oil and xylol. Instead, however, of transferring the material into paraffine and making sections, pieces of it were placed on a slide and crumbled. Alcohol was added to make a suspension, which was smeared and fixed. These smears were again washed with 95% alcohol to remove the xylol, with water to remove the alcohol and stained by Burke's Modification. More than 95% of the organisms were now found to be entirely gram-negative, a few were partially decolorized and only an occasional gram-positive form was seen. It is clear, therefore, that hardening and fixation alone may so alter gram-positive organisms present in tissues that they are no longer capable of retaining the stain. The observation calls attention to a source of error in examining tissue for the presence of gram-positive organisms. It may also account for an idea which prevails, to the effect that the animal body alone is capable of changing the gram reaction of injected organisms. In the experiment last quoted, the organisms in smears from the tissue made just after death, were still sharply gram-positive, and had not been affected in this respect by their residence in the animal body. It was only after they had been through the processes to which tissue to be cut is submitted, that they became gram-negative.

[1] Churchman, J. W., *J. Exp. Med.*, 1927, xlvi, 4007.

3845

Studies in the Agglutination Prozone.

GERALD S. SHIBLEY.

From the Department of Medicine of the College of Physicians and Surgeons of Columbia University, and the Presbyterian Hospital.

In the course of studies of the mechanism of agglutination, we have sought to explain the pro-agglutinoid zone. Agglutinative sera showing a natural prozone have been so rare in our experience that we have resorted to the study of the prozone in serum produced artificially by heating.

The following is a summary of results observed with such "heat" prozones. Horse and rabbit sera were used. The temperature used for securing this type of prozone is usually stated to be 70° to 75° C. Our observations have shown that with certain sera, temperatures ranging from 56°, or possibly lower, to 76° C. are effective (at pH's 5.0 to 7.0), longer heating being required at the lower temperatures, *e. g.*, 3 minutes at 68° produces a prozone, while 2 to 4 hours is necessary at 60°. Heating to 76-78° destroys all agglutinin. In the case of some of the typhoid sera, the production of a prozone was accompanied by a faint opalescence. Centrifugalization failed to clear the serum but passage through Berkfeld V candles was successful. Accompanying this removal of turbidity, the prozone disappears and coincidentally the titre of the the serum falls (Table 1).

TABLE I.
Effect of Berkfeld filtration upon the prozone serum (R 44).

Sera	*Agglutination*							
	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
Untreated serum	c	c	c	c	c	c	c	c
Heated (prozone) serum	—	—	—	—	—	c	c	c
Filtered prozone serum	c	c	c	c	c	c	—	—

c = complete agglutination; — = no agglutination.

In the case of a dysentery prozone serum not showing this turbidity a similar eradication of prozone and coincident reduction of titre was obtained by adding kaolin.

When prozone serum (typhoid) was added in proportions of 1:1, 2:1 and 4:1 to an untreated *B. melitensis* serum no inhibition of agglutination of the melitensis organism occurred, *i. e.*, typhoid prozone serum does not produce a prozone in melitensis serum. Also, in the case of closely related bacteria (dysentery strains), the

addition of Shiga prozone serum to Flexner, Mt. Desert, "Y" and Sonne B sera in similar fashion failed to produce prozones. In other words, the prozone effect seems to be highly specific. Prozone serum, 1/20, showing no agglutination, when treated with excess bacteria, shows typical absorption of agglutinin. When this absorption process is carried out with carefully graded doses of bacteria, the prozone goes first, *i. e.*, there would seem to be absorption first of the inhibitory factor and then of normal agglutinin.

As serum is exposed to increasing heat (*B. dysentery*, Shiga), first the prozone appears, then it is removed and finally all agglutinin is destroyed. Table II illustrates this effect.

TABLE II.

Effect of varying heat upon B. Dysentery, Shiga, serum.

Temperature of heating 6 min.	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
Unheated	c	c	c	c	c	c	c	c	3
60°	c	c	c	c	c	c	c	c	3
62°	c	c	c	c	c	c	c	c	3
64°	±	±	c	c	c	c	c	c	±
66°	—	—	—	c	c	c	c	c	±
68°	—	—	—	c	c	c	c	c	—
70°	±	±	±	c	c	c	c	3	
72°	c	c	c	c	c	c	2	—	
74°	c	c	c	c	c	c	2	—	—
76°	±	±	±	—	—	—	—	—	—
78°	—	—	—	—	—	—	—	—	—

c = complete agglutination, clear supernatant fluid.
3 = heavy flocculation, supernatant fluid not clear.
2 = visible granular appearance.
1 = visible granular appearance with hand lens.
— = no agglutination.

The zone begins at 64°, reaches a peak at 66-68° and fades at 72° C.; coincident with the disappearance of the zone there is a corresponding drop in titre. This finding taken in conjunction with the reduction of agglutinin titre accompanying prozone removal by filtration or by kaolin treatment supports the assumption that the prozone factor is modified agglutinin; in favor of this also is the evidence of absorption of agglutinin in the prozone.

The cataphoretic behavior of bacteria treated with prozone sera in the inhibition dilutions very closely resembles that of bacteria treated with unheated serum. This suggests that there is union in this zone between organisms and modified agglutinin; the absorption experiments above are further proof of this.

We have shown that sensitization of bacteria is selective coating by the globulin of the antibody and that flocculation follows because the coated bacteria now take on the character of particles of denatured globulin. It is probable that the union between organism and modified agglutinin, *i. e.*, film formation, in the prozone, is incomplete because of the modification of agglutinin noted above, and by the same token that flocculation cannot follow. If we accept this explanation it is necessary to assume that in the lower dilutions, where modified and unmodified agglutinin are present together, the former (agglutinoid of Ehrlich) has a greater affinity for the bacteria; this is the original hypotheses set forth by Ehrlich. In support of this there is the selective absorption referred to above.

3846

Critical Concentrations of Bioses.

GEOFFREY L. PESKETT. (Introduced by Walter H. Eddy.)

From the Laboratories of the Department of Biochemistry, Oxford University, England, and the Department of Physiological Chemistry, Teachers' College, Columbia University.

The observations on yeast growth which have been recorded by numerous workers, together with the results reported here, seem to require some extension of the theory of "bios" originally stated by Wildiers,[1] *viz.*, "That a substance of unknown composition, or bios, is indispensable for the development of yeast." For the development of some species of yeast at least, a certain minimal concentration of a substance or substances is indispensable. Moreover, the following results show that this minimal concentration may be very critical, *i. e.*, a given yeast species may be very sensitive to a small diminution in the concentration of bios below the minimum value.

Critical Concentration of Bios. The following type of experimental result has led to the postulation of a theory of "critical" concentrations. A series of 7 different concentrations of "bios" (in the form of a 70% alcoholic extract of autolyzed yeast, from which the alcohol had been evaporated) in a basal salt-sugar medium, was inoculated with varying quantities of yeast cells. After 5 days' incubation at 25° C. a concentration of cells (constant within 22%) was observed corresponding to each "bios" concentration—except the smallest. In the latter case (0.025 mg. of bios per cc.) this constancy was absent, and the smaller seedings attained a final concen-

tration which was 72% less than that with the largest seeding. The figures are given in Table I.

TABLE I.

Bios concentration (mg. per cc.)	Cell concentration found after 120 hrs. (millions per cc.).			
	Seedings 2.0 millions	0.4 millions	0.2 millions	0.04 millions
1.45	87.6	84.8	82.0	88.0
0.73	70.5	67.0	58.4	54.8
0.36	44.5	43.8	43.8	43.1
0.18	42.4	42.4	42.0	38.1
0.09	40.0	38.0	37.0	36.0
0.045	25.6	21.2	20.8	20.1
0.025	20.1	10.1	5.6	5.6

If the concentration of bios falls below a certain minimum (in the above experiment .045) then there is a marked difference between the behavior of large and small seedings. Further investigation is necessary before any explanation of this effect can be given. However, the determination of the minimum or "critical" concentration for any given preparation of bios may be made a simple means of gauging its potency. Such a determination measures the ability of a preparation to enable *small* inoculations to grow—a criterion of true bios activity as implied by Wildiers.

Obviously many factors may influence the determination of crit-

TABLE II.
Sensitivity of yeast to changes in concentration of bios.

Nos.	Bios concentration* (mg. per cc.)	Concentration of cells after 6 days at 25° C. (millions per cc.)			
		Seedings	840,000 cells	3,000 cells	Difference per cent
1	0.041	(Volumes constant at 5cc.)	10.1	11.0	8.9
2	0.038		12.4	11.7	5.7
3	0.036		11.7	9.9	15.4
4	0.033		11.0	6.0	45.5
5	0.030		10.6	9.2	13.2
—	—		—	—	—
6	0.027		9.6	7.8	18.8
7	0.027		9.2	2.6	71.7
8	0.025		9.9	2.6	73.5
9	0.022		8.9	2.7	69.7
10	0.019		11.0	1.2	89.1
11	0.016		12.8	5.6	56.3

*The different concentrations of "Bios" were obtained by adding to the basal medium a constant volume of "Bios" solution. The latter was diluted successively 5 times to prepare Nos. 1 to 6 inclusive. A fresh solution was used and successive dilutions of it made in preparing 7 to 11. This possibly explains the discrepancies between Nos. 6 and 7.

ical concentration. Activity cannot as yet be measured in terms of absolute units. If, however, the principle of "critical" concentrations is sound—and the experiments reported seem to show that it is—then it is possible, by keeping the conditions of test uniform, to determine the relative potency of a series of preparations. The method described above, furnishes a means of testing the activity of isolated fractions.

Preliminary studies of some factors which may influence critical concentration values have yielded the following results. (See Table II and seq.)

The experiment reported in Table II was continued to the 19th day. The percentage differences between the concentration of cells attained by the 2 seedings after 19 days were as follows:

Medium No.	1	2	3	4	5	6	7	8	9	10	11
% Diff.	10.7	14.5	10.7	20.3	12.9	3.4	33.2	24.9	35.2	56.2	30.4

Reference to Table II shows clearly that if the concentration of Bios is above .027 the final cell production is practically independent of the seeding. Below .027 marked differences appear. .027-.030 is therefore the approximate critical concentration for this bios preparation.

Is Volume of Medium a factor in critical concentration determination? Two tubes of 5 cc. and a flask of 1500 cc. of basal medium containing 0.045 mg. "bios" per cc. were each seeded with 3,000 cells. After 10 days, but not earlier, the concentration was 16 million cells per cc. in each of the 3 cultures. In spite of the difference in volumes and hence in cells per cc. (2 cells per cc. in one case and 600 per cc. in the other 2) no difference in final cell concentration is detectible provided critical concentration of bios is present.

TABLE III.

	Bios concentration (mg. per cc.)	Time days	Cell concentrations attained (millions per cc.) Seeding 500,000	
A. Alpha-Bios*	0.025	9	6	
,,	0.1, 0.05	9	3-4	
,,	0.0125 & 0.006	9	3-4	
But at a seeding of 5,000 cells and .025 bios the crop was only 1.				
			Seeding 500,000	5,000
B. Beta-Bios†	0.1	7	16	—
,,	0.075	7	14	15
,,	0.05	7	12	6
,,	0.025	7	4	—
This result indicates .05-.075 as the critical concentration of Beta-Bios.				

*Prepared by R. W. Kerr after method Eddy, Kerr and Williams.[2]
†Reported by R. W. Kerr in this Journal.[3]

Following the above methodology various bios preparations have been tested to establish critical concentrations. (See Table III.)

In the case of Alpha-bios no critical concentration was established, but with 0.025 mg. per cc. the highest growth occurred. In higher concentrations there was some inhibition of growth. The critical concentration of Beta-bios was 0.075 to 0.05 mg. per cc., so that its activity is only one-half that of a crude alcoholic extract of autolyzed yeast, (see above, critical concentration of 0.03 to 0.025 mg. per cc.). This confirms the results obtained in Dr. Eddy's laboratory and elsewhere and supports the view that several factors of the bios type are involved. Tests are in progress on Kerr's synthetic bios. Indications to date are that a critical concentration is demonstrable, but the limits are not yet determined.

Discussion. The results reported here find confirmation in the work of Clark,[4] who found that a maximum constant crop is obtainable that is independent of the size of seeding; presumably the concentrations of wort he employed were all larger than the critical concentration value. The type of curve obtained by Eddy, Heft, Stevenson and Johnson[5] for the yeast stimulatory power of alfalfa extract indicated that a certain maximum effect was obtained with a certain concentration of extract, and that higher concentrations have no greater effect. It seems probable that here the optimum effect was with a "critical" concentration of alfalfa extract bios. The principal advantage of the "critical" concentration method of study is that it takes account of effect on small seedings compared with effect on large seedings, and therefore measures the "true bios activity" in the sense of Wildiers, *i. e.*, the power of enabling small inoculations to grow.

Summary. A study of the effect of varying simultaneously the size of seeding and the concentration of bios, shows that a "critical" concentration of bios can be defined, in the presence of which a small seeding will attain the same cell concentration as a large seeding; while a diminution of the bios concentration below the critical value (by as little as 0.005 mg. per cc.) has been recognized by the large divergence in the cell concentrations attained. The "critical" concentrations of some crude yeast extract, purified Alpha and Beta bioses have been studied.

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[1] Wildiers, *La Cellule*, 1901, xviii, 313.

[2] Eddy, Kerr and Williams, *J. Am. Chem. Soc.*, 1924, xlvi, 2846.

[3] Kerr, R. W., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 344.

[4] Clark, N. A., *J. Phys. Chem.*, 1922, xxvi, 42.

[5] Eddy, Heft, Stevenson and Johnson, *J. Biol. Chem.*, 1921, xlvii, 249.

3847

The Isolation of a Beta Bios.

RALPH W. KERR. (Introduced by Walter H. Eddy.)

From the Laboratory of Physiological Chemistry, Teachers College, Columbia University.

In 1924 Eddy, Kerr and Williams[1] reported the isolation of a crystalline Bios M.P.223. As tests of the physiological activity of this product proceeded, it became evident that this substance alone could not account for all the yeast growth stimulatory power of its source, yeast autolyzate. Search was therefore begun for another bios and for convenience Bios M.P.223 will be referred to hereafter as Alpha-bios. By manipulation of the yeast autolyzate it has now been possible to fraction it into at least 3 specifically distinct bios-containing fractions. One fraction yields a homogenous product not hitherto described and which is here designated as Beta-bios. A third fraction yields a concentrate whose behavior toward precipitants suggests Lucas and Miller's Bios II[2] and which we will call here Gamma-bios.

The evidence of the existence of Beta-bios which led to the development of the fractionation methods for its isolation was first obtained by studying the behavior of yeast autolyzate and Alpha-bios solutions under electrodialysis. Using a 14 compartment carbon cell with parchment paper septa and yeast autolyzate, yeast stimulatory activity was sharply concentrated in 2 separate compartments of quite different pH. No such separation occurred when the cell was filled with pure Alpha-bios solution.

The detailed method of fractionation will be reported later. The essential feature of the separation of Beta-bios is the formation of an insoluble barium salt of this substance preliminary to the separation of Alpha and Gamma bioses. This barium salt is then decomposed with sulfuric acid. Basic impurities are eliminated by silver and mercuric sulfate and phosphotungstic acid. Non-nitrogenous acids are removed by extraction with acetone.

The Beta-bios isolated by our procedure is very hygroscopic. When dehydrated with acetone and the aid of the vacuum desiccator it is a fine white granular powder decomposing at 100° C. on prolonged heating. It dissolves to form a colorless solution in acids such as HCl, but takes on a bright yellow color in neutral solvents such as water and an intense yellow turning to brown in alkalies. It gives the following reactions:

Molisch test = negative. Fehling-Benedict = negative even after boiling 10 hours with 10% HCl.

Ninhydrin test = positive. Folin phenol test = positive. Millon test = doubtful.

Ppt. by basic lead acetate but not by normal lead acetate.

Indol test negative but positive after fusion of product with KOH.

Decomposed at 110° C. with evolution of CO_2.

Fused with KOH evolves ammonia and skatole, the latter even when fused in copper tube with an atmosphere of hydrogen.

Does not ppt. with phosphotungstic acid.

S, P, Halogens and metals absent.

Quantitative elementary analysis:

	%C	%H	%N*	
Product 1	42.76	6.52	6.18	5.47
Product 2	42.58	6.42		
Product 3	42.65	6.60		

* By micro-Kjeldahl.

Evidence of homogeneity of product rests to date on the fact that repeated isolations have always produced a compound with identical physical, chemical and physiological properties and constancy of elementary analysis. At present we are investigating a synthetic compound built upon the theory of Beta-bios construction. This synthetic product to date exhibits physiological activities similar to that of Beta-bios but details of this work are reserved for a later communication.

The physiological activity has been studied to determine critical concentration;[3] effect on 6 different pure strains of yeast; and effect of combination with different culture media such as Clark's, Nageli's, Medium F, etc. The effect varies somewhat with different yeast and culture media but at a conc. of .05-.075 milligrams per cc. maximum effects are demonstrable amounting to at least ten fold growth in 48 to 72 hours incubation at 31° C. In these tests the Funk-Dubin method[4] was used, and in most tests R. J. Williams' culture medium.[5]

[1] Eddy, Kerr and Williams, *J. Am. Chem. Soc.*, 1924, xlvi, 2846.

[2] Lucas, G. H. W., *J. Phys. Chem.*, 1924, xxviii, 1180.

[3] Peskett, G. L., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 340.

[4] Funk and Dubin, *J. Biol. Chem.*, 1920, xliv, 487.

[5] Williams, R. J., *J. Am. Chem. Soc.*, 1927, xlix, 227.

3848

Measurement of Hydrogen Ion Concentration by Means of the Glass Electrode.

M. L. ANSON AND A. E. MIRSKY. (Introduced by Alfred E. Cohn.)

From the Department of Animal Pathology of the Rockefeller Institute and from the Hospital of the Rockefeller Institute.

Acidity plays such an important rôle in modern biology that the methods of measuring it are of considerable interest. A method has recently been introduced that is admirably adapted to many needs of experimental biology. The glass electrode is as accurate as the hydrogen electrode, as rapid as the quinhydrone electrode, and (once the apparatus has been set up) as easy to use as colorimetric methods. It can, moreover, be used under conditions when no other method is applicable—for example, for the direct measurement of the pH of whole blood.

The glass electrode was gradually developed over a period of fifty years by a number of investigators (including Lord Kelvin,[1] Cremer[2] and Haber[3]); its recent application to the exact measurement of hydrogen ion concentration is due to Kerridge.[4] We have used it for this purpose, but to obtain satisfactory results we find it necessary to take a precaution that is hardly mentioned in previous papers on the glass electrode. At the same time, we have introduced several modifications that simplify and shorten the manipulations.

One form of electrode consists of a glass tube, one end of which is blown to a very thin bulb, a depression in which forms the membrane. If on the 2 sides of the membrane are placed 2 solutions of different pH, a potential difference is set up, and under certain conditions, this potential is determined by the difference in pH of the 2 solutions. Consequently, if the pH of one solution and the membrane potential are known, the pH of the other solution can be calculated. For the measurement of potential the current is led off from each solution by means of a KCl bridge to a calomel half cell. The difficulty we have encountered is concerned with the KCl, for if it contaminates the solutions, variable and irreproducible potentials are obtained. Contamination does not occur by diffusion, but simply because a saturated KCl solution is relatively heavy and tends to flow down. An obvious way, therefore, of avoiding the difficulty is to introduce the KCl from below, and this we have done. It is often convenient, however, to dip the bridge in from above. To avoid contamination, we attach to each bridge a tip with a very

fine opening and containing KCl in agar. An even simpler way of "avoiding" KCl contamination in the solution of *known* pH is to prepare it saturated with KCl, in which case this bridge can be left in permanently. A further step is to seal the glass electrode to the calomel half cell and thus dispense with this KCl bridge. By this manoeuvre the half cell and the electrode form one piece.

After a little practice it is easy to blow the electrode oneself. In the construction of a stand, the arms which support the electrode and calomel cells are made of amber for insulation, but we find quartz more convenient. The only instruments one need purchase are a potentiometer of the usual type and an electrometer. We have used the Lindemann electrometer supplied by the Cambridge Instrument Company. The rest of the apparatus is easily made.

[1] Thomson, W., *Proc. Roy. Soc. London*, 1874-75, xxiii, 463.

[2] Cremer, M., *Z. Biol.*, 1906, xlvii, 562.

[3] Haber, F., und Klemensiewicz, H., *Z. physik. Chem.*, 1906, lxvii, 385.

[4] Kerridge, P. M. T., *The Biochem. J.*, 1925, xix, 611, and *J. Scientific Instruments*, 1926, iii, 404.

3849

Changes in Lactic Acid and Glucose in the Blood on Passage Through Organs.

H. E. HIMWICH, Y. D. KOSKOFF AND L. H. NAHUM.

From the Department of Physiology, Yale University.

The following research was undertaken to determine the factors regulating the concentration of lactic acid in the blood of mammals. Observations were made on 32 dogs most of which were decerebrate. The amount of lactic acid in the arterial blood was compared with that of the blood draining the liver, spleen, portal system, kidney, testicle, lower extremities, brain, thyroid, lungs and heart. Lactic acid was estimated by the method of Shaffer, Cotonio and Friedemann. Sugar was determined by the Shaffer-Hartmann method. A difference of 5 mg. % or more between the lactic acid content in the arterial and venous samples was considered significant. Typical results are found in Table I, where the muscle poured lactic acid in the blood stream and the liver removed it. Thus, in 27 of 51 observations the muscles added to the lactic acid content of the blood passing through them. In 19 cases the difference between arterial and venous blood was not considered signifi-

cant, and in 5 instances only did the muscles remove lactic acid. On the other hand, in 21 of 34 observations the liver removed lactic acid. However, in 6 instances the liver was adding lactic acid to the blood and therefore could not explain the lower arterial level. It is clear that some organ other than the liver was removing lactic acid. Results from many of the organs were not constant. Sometimes they would remove lactic acid from the blood though in decerebrate animals they usually added lactic acid.

TABLE I.
Changes in lactic acid and glucose in arterial blood and blood draining muscles, portal system and liver.

Date	Lactic acid mg. %				Glucose mg. %			
	Femoral Artery	Femoral Vein	Portal Vein	Hepatic Vein	Femoral Artery	Femoral Vein	Portal Vein	Hepatic Vein
Aug. 25	84.2	102.7	80.3	70.2	187	175	178	227
Dec. 3	88.6	113.2	88.7	65.8	166	162	157	174
Dec. 14	80.5	99.1	77.4	67.6	75	73	71	90

TABLE II.
Changes in lactic acid content of blood passing through the heart.

Date	Lactic acid mg. %	
	Femoral artery	Coronary vein
Dec. 22	61.8	55.3
	52.9	44.5
Jan. 17	41.0	30.2
Jan. 20	83.1	60.2
	131.8	117.4
Jan. 31	62.1	57.0

We next studied the heart. As seen in Table II the heart, in 6 observations, was removing lactic acid from the blood passing through it. Even small differences are significant in the heart because of its relatively large vascular supply.

Not only lactic acid but also glucose was determined on the blood samples. The organs with the exception of the liver usually removed glucose from the blood while the liver poured out increased amounts into the blood stream. There appears to be a cycle:

$$\text{Liver} \underset{\text{Lactic acid}}{\overset{\text{Glucose}}{\rightleftarrows}} \text{Muscle}$$

Summary. Changes in lactic acid and glucose content of the blood through organs have been studied in 32 dogs. The main source of lactic acid is the muscle while the organ chiefly concerned with its removal is the liver. The heart may participate in the removal of lactic acid from the blood. Since all the organs, with the exception

of the liver, remove glucose from the blood and the liver adds glucose there seems to be a carbohydrate cycle between muscle and liver, muscles sending lactic acid through the blood to the liver and the liver returning glucose to the muscles.

3850

Difference in Calcium Level of the Blood Between the Male and Female Cod.

A. F. HESS, C. E. BILLS, M. WEINSTOCK AND H. RIVKIN.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

As far as we are aware, no reports have been made of calcium in the blood of fish. In the course of a study of various physiologic phenomena in the cod, a marked difference was found in the level of calcium in the blood of the male and the female. The percentage in the former was found to be approximately 9 to 12.5 mg., the cause of the variability not being ascertained. In the female a percentage as high as 29 was not uncommon, the lowest figure being 12.7 mg. These variations were due clearly to the generative state of the fish. When the roe was large and mature, the serum calcium percentage was high, whereas when the roe was hard, or after the fish was spent, the percentages were markedly lower. A similar relationship between spawning season and calcium level of the serum was found to exist in the puffer fish. Calcium in the female was found as high as 26 mg. %, whereas in the male it was about 12 mg. %. This phenomenon is not, however, common to all fish. For example, in the dog fish, a viviparous species, the calcium level was high in both male and female.

The inorganic phosphate was far more constant than the calcium. Generally it ranged between 9 and 12 mg. %, the former amount having been obtained in a female cod which had 17.8 mg. % of calcium in the blood. The highest figure was 14.7 in a female with 13.3 mg. % of calcium. From these figures, it is evident that the inorganic phosphorus does not bear a definite ratio to the percentage of calcium. Nor does it vary with the spawning season, a fact which is emphasized by an instance in which a female with a calcium percentage of 29 mg. in the blood had a percentage of inorganic phosphorus of only 10.9 mg.

The total cholesterol content of the blood varied within wide

limits. Figures as high as 319 and 330 mg. % were found in 2 females which were spent. The blood sugar also showed no distinctive relationship.

The function of the high blood calcium in fish cannot be the formation of exceptionally strong bone. On the other hand, the eggs were found especially rich in calcium, which suggests that the calcium may be required for their elaboration. In connection with this study, attention should be called to the observation of Riddle[1] and others to the effect that the blood calcium increases markedly in birds at the period of ovulation. This has been attributed to the requirement incidental to the formation of the shell.

[1] Riddle, O., and Reinhart, W. H., *Am. J. Physiol.*, 1926, lxxvi, 660.

3851

Synthesis of Racemic 2, 5-Dihydroxy Phenyl Alanine (Gentisic Alanine)

LOUIS FREEDMAN.

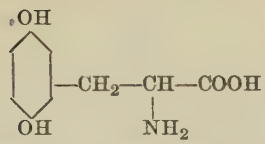
From the Research Division of the H. A. Metz Laboratories, Brooklyn, N. Y.

In 1922, in cooperation with Dr. Casimir Funk, we attempted the synthesis of the gentisic alanine by the Sasaki[1] and Hirai[2, 3] methods, namely condensation of dimethoxy gentisic aldehyde with glycine anhydride in presence of anhydrous sodium acetate and acetic anhydride, and subsequent splitting of the diketo piperazine compound by means of prolonged heating with strong hydriodic acid and red phosphorus.

We failed to obtain the desired compound at that time and, due to pressure of other work, temporarily dropped the problem. Early in 1927, we again resumed this work and succeeded in preparing a crystalline substance which gave all the reactions of the desired compound. A Kjeldahl nitrogen determination gave the correct analysis for nitrogen, but due to the small amount of material available, we were unable to do a complete combustion at that time and were therefore loathe to publish our results.

As K. Hirai[4] has lately reported the synthesis of this amino acid, and as our compound appears to be identical with Hirai's in all respects, we feel justified in reporting our work.

Splitting of the 2.5-dimethoxy benzal glycine anhydride to racemic 2, 5-dihydroxy phenyl alanine.



1.1 gm. of 2.5-dimethoxy benzal glycine anhydride were mixed with 10 cc. hydriodic acid (s.g. 1.7) and 0.8 gm. red phosphorus and the mixture refluxed in an oil bath at 110° C. for 7 hours. The mixture, on cooling, was diluted with water, filtered and the filtrate acidified with glacial acetic acid. 20% lead acetate solution was added to complete precipitation. The precipitates of lead iodide and lead phosphate were filtered and to the clear colorless filtrate, ammonia was added until a precipitate was no longer formed. This casein-like precipitate which is the lead salt of the amino acid was filtered and washed well with water.

The lead salt was suspended in water and decomposed with hydrogen sulfide gas. The precipitate of lead sulfide was filtered, the excess hydrogen sulfide removed by a stream of CO_2 gas and the clear filtrate was evaporated to dryness in vacuum under CO_2 gas.

The crystalline residue was taken up in a small amount of water containing a trace of sulfur dioxide gas and the solution allowed to stand in a vacuum dessicator over sulfuric acid. Beautiful ten-sided prisms, probably rhombic, formed. These crystals weighed 0.23 gm. On recrystallization from water containing a trace of sulfur dioxide, 0.12 gm. of the decahedrons was obtained.

The compound melted at 204 to 205° C. (uncorrected), with decomposition. It was readily soluble in warm water, slightly soluble in cold water and insoluble in most organic solvents. Its aqueous solution gradually turned to a reddish brown on addition of ammonia. With a drop of ferric chloride solution, it gave a greyish-green color which gradually turned dark and finally gave a black precipitate on standing.

It reduced an ammoniacal silver nitrate solution very quickly in the cold. It also gave a positive ninhydrin reaction for presence of a free α-amino group.

Nitrogen analysis — Found —7.26%.
Calculated for $C_9H_{11}O_4$ —7.10%.

Although this amino acid has not been found in nature, it is of special significance from a biochemical standpoint, since its structure leads to the hypothesis that it may be a possible precursor of hemogentisic acid in alkaptonurics.

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[1] Sasaki, T., *Ber. d. deutsch. chem. Geselsch.*, 1921, liv, 163.

[2] Hirai, K., *Biochem. Zeit.*, 1921, cxiv, 67.

[3] Hirai, K., *Biochem. Zeit.*, 1926, clxxvii, 449.

[4] Hirai, K., *Biochem. Zeit.*, 1927, clxxxix, 88.

3852

Use of Hydrogen Peroxide for Organic Oxidations.

FRANCES KRASNOW AND A. S. ROSEN.

From the Laboratory of Biological Chemistry of Columbia University at the College of Physicians and Surgeons.

In the use of hydrogen peroxide to facilitate micro-organic oxidations, we encountered certain difficulties and feel that our experience may be of value to other investigators.

Hydrogen peroxide, though well adapted for oxidation generally, cannot be used in the determination of organic phosphorus. The market products very frequently contain phosphate or one of the other substances giving like reactions. Of 6 samples tested within the last 2 years, 4 contained considerable amounts of this substance and 2 contained traces. Using the Tisdall method[1] the following results were obtained:

Sample No.	Description	Phosphate Content mg. per 100 cc.
1.	Superoxol	0.03
2.	Perhydrol	trace
3.	Superoxol	2.7
4.	Superoxol	2.8
5.	Hydrogen peroxide Solution of Hydrogen Dioxide.	1.5
6.	"	1.8

When the strychnine molydate solution is added to diluted superoxol or to the solution of hydrogen dioxide, no precipitate is obtained. This is not due to dilution of the phosphate content. If 50 cc. of the hydrogen dioxide solution is evaporated (water bath) nearly to dryness and the residue dissolved in 5 cc. H_2O, no precipitate results. However, when it is evaporated until completely dry and the residue then dissolved in 5 cc. of water, a very heavy precipitate quickly separates out. Superoxol (2 cc.) evaporated to dryness shows a phosphate content of 0.032 mg. which is equivalent to 1.6 mg. per 100 cc. After digestion, 2 cc. of the same preparation yields 2.7 mg. per 100 cc. There seems to be some substance in the superoxol, as well as in the solution of hydrogen dioxide, that interferes with the precipitation of phosphate.

[1] Tisdall, F. F., *J. Biol. Chem.*, 1922, l, 329.

3853

Growth of the Lobster, Homarus Americanus.

ARATA TERAO. (Introduced by Raymond Pearl.)

From the Institute for Biological Research of The Johns Hopkins University.

Among the workers on the growth of the lobster, credit is due to Hadley[1] for giving the best data yet available. The object of the present paper is to analyze Hadley's data mathematically and make some comments upon it.*

It should be borne in mind that there remains something to be desired in Hadley's data. First of all, the material for the females is relatively poor, not only in deficiency of larger specimens but also in the sense that the growth curve abruptly shows linearity immediately after reaching the sexual maturity, if one assumes his time basis to be correct. And yet it is almost beyond doubt that his record of the males covers all the stages up to the oldest one, although his estimation of the age coupled with actual observation is trustworthy up to 6 years only.

It should first be noted that it is impossible to get by mathematical analysis any definite idea about the true age of the larger specimens out of the data at our disposal. First, taking all of Hadley's estimates of the age of the males for granted, a logarithmic parabola of the form,

$$y = a + bx + cx^2 + d \log (x - a)$$

was fitted by least squares. The constants, shifting the origin at —1, were as follows:

$$y = -739.62 + 0.67x - 0.0039x^2 + 562.03 \log (x + 20) \qquad (1)$$

where y represents the length of the males in millimeters and x the age of the same in 60-day units. On the other hand, a logarithmic parabola fitted to the observations on the males up to 6 years (*i. e.*, over the period of definite observation as distinguished from estimates of age) gave the following result:

$$y = 2.51 + 5.69x - 0.00098x^2 + 38.44 \log x \qquad (2)$$

where x and y represent the same variables as in (1) with the origin at —1. The maximum age of the males obtained from the equation (1) is 35.196 years, whereas the largest males, measuring 568 mm. in length, would be less than 15 years old in case their growth followed curve (2) further than 6 years.

* I am greatly obliged to Dr. John R. Miner for help in the matter of computation of the constants.

Turning now to another possible way of approaching the problem we may consider the lengths of the males in reference to the stages (ecdyses) equally spaced, there is seen to be an unmistakably logistic trend to the curve, as shown in Fig. 1, though in detail the fit is not particularly good. Computing the approximte values of the constants, we have

$$y = \frac{650}{1 + e^{4.38-0.178x}} \qquad (3)$$

where y represents the length of the males in millimeters and x the ordinal number of the stages. The observed and calculated values of y are given in Table I.

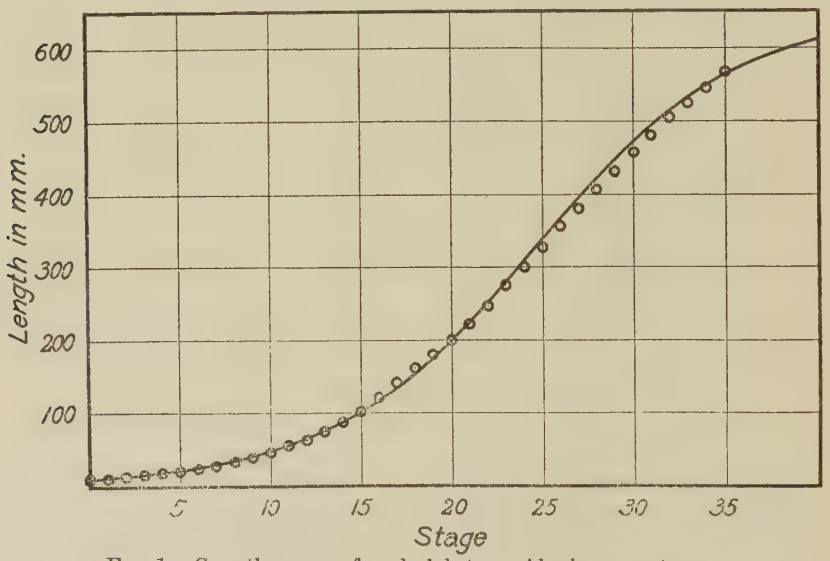

FIG. 1. Growth curve of male lobster. Abscissae = stages.

In this curve the abscissae represent biological rather than temporal age. What the time intervals are between the successive developmental stages is not known except for the early stages, but what is most certainly known is that they are not equal throughout the whole period of growth. In short Fig. 1 and Table I have no definite, known relation to time whatever. They represent growth only in terms of biological age. What the results show is that if it be assumed that each successive moult of a lobster represents an equal advance in its biologic age, the growth of the body in length follows approximately a logistic curve. Over the period of definite observations of temporal age (6 years) the growth in length of body does not follow a logistic course when plotted against time,

TABLE I.
Growth of the Lobster.

Stage x	Observed y	Calculated y	Stage x	Observed y	Calculated y
0	8.2	8.02	18	162.0	153.81
1	9.6	9.55	19	180.0	175.72
2	11.4	11.39	20	200.0	199.51
3	13.5	13.57	21	222.0	224.99
4	16.0	16.16	22	247.0	251.90
5	18.8	19.23	23	275.0	279.92
6	22.5	22.74	24	300.0	308.63
7	26.5	27.13	25	327.0	337.60
8	32.0	32.16	26	356.0	366.37
9	37.9	38.08	27	380.0	394.50
10	45.0	45.00	28	406.0	421.57
11	53.0	53.07	29	431.0	447.26
12	62.0	62.44	30	457.0	471.28
13	73.0	73.26	31	480.0	493.44
14	86.0	85.69	32	505.0	513.66
15	102.0	99.85	33	525.0	531.89
16	121.0	115.88	34	546.0	548.17
17	141.0	133.85	35	568.0	562.39

but instead a logarithmic parabola. For this difference in the form of the growth curve in the lobster, when body length is plotted against (a) temporal and (b) biologic age, I have at present no explanation, but the fact seems worthy of record.

[1] Hadley, Philip P. 1906. 36th Ann. Rep. Comm. Inland Fish, Rhode Island, 153-226, pls. xxvi-xxxvii and xl. The same data are quoted in Herrick, Bul. U. S. Bur. Fish., 1911, xxix, 747.

3854

Study of Alpha and Beta Units of an Anti-Paratyphoid Bacteriophage.

PHILIP HADLEY AND EUGENIA DABNEY.

From the Hygienic Laboratory, University of Michigan, Ann Arbor.

Under the influence of a fresh, sewage filtrate we isolated a lytic agent for an old laboratory strain of *B. paratyphosus A,* in the S cyclostage. This filtrate, which was first active in F_3, was enhanced to a high titer by alternate feeding and filtration in series. When tested by the plate method against the homologous culture there first appeared only large plaques. Later (F_6), the filtrate gave small plaques in addition to the large. With continued propagation there was a tendency for the large plaques to be replaced by the small.

The large lytic areas possessed an average diameter of 6 to 7 mm. when mature. As a rule, there were no areas of intermediate size. A few that measured from 3 to 4 mm. possessed the chief characteristics of the large plaques, and on further propagation they always reproduced the large area type. The small lytic areas were sharp and clearcut, while the large areas were usually surrounded by a hazy zone of imperfectly lysed culture. As a rule the principle determining the large areas permitted the appearance of a relatively larger number of secondary, resistant colonies than did the small-area principle. Frequently, under conditions of crowding, the large areas enclosed one or more of the small. In this case the latter were especially distinct if they happened to lie in the marginal zone of the large areas; for, here, the small areas completed the lysis which had been left imperfect by the large areas.

Separate, pure line, lytic filtrates were prepared through isolations performed upon discrete large and small areas respectively, and these filtrates were built up to a moderately high titer by alternate feeding and filtration in series. The large area principle will hereafter be termed the α principle and the small area principle will be termed the β. The large and the small areas will receive the same respective designations (α and β). When the alpha filtrate was tested against the homologous S type culture it gave only large (α) areas. Such areas were reproduced constantly through 15 plate-generations by the following method: A straight needle, moistened in sterile broth, was touched lightly to the center of a discrete area, then dipped in a tube containing 5 cc. of sterile, beef infusion broth of pH 7.8. One loop (4 mm.) of the broth was then spread over the surface of an agar plate previously seeded with 3 drops of a young broth culture of the original strain. The number of lytic areas arising on such a plate, after an incubation of 24 hours or less, was usually 10 to 100. After the pure-line alpha filtrate had been propagated in broth by alternate feeding and filtration through 6 to 8 series, the β principle made its appearance and registered on agar plates by the production of small areas in addition to the large. In other words, the α pure-line principle did not "breed true."

When β filtrate was tested on plates against the S type culture only small (β) areas were produced. This principle was not propagated by "colony-to-plate" transfers as in the case of the α principle; but when the filtrate was propagated by alternate feedings and filtrations for 12 generations it still produced only the β areas. This has also been the experience of one of the writers

working with a β principle of the Shiga dysentery bacillus. The pure-line β principle has thus appeared to "breed true." Moreover the β principle of the Shiga bacillus has been found to retain its characteristics after being sealed in glass ampoules over a period of 3 years.

The points of thermal inactivation of the 2 principles were roughly ascertained. In this connection it may be recalled that different investigators have reported the thermal "death point" of the bacteriophage at various levels—from 62 to 74° C. A fraction of a cc. of each principle was taken into a thin, drawn-out capillary tube and the tips sealed 2 inches from the ends of the column. The capillaries were then immersed in a water bath and heated at various temperatures. After cooling, the contents were expelled on agar plates previously seeded with 3 drops of the S type culture. If lysis did not occur it was assumed that the heated principle had been inactivated. No attempt to cause the regeneration of a "latent" principle was made. By means of these tests it was ascertained: (1) that the α principle was rendered inert by 30 minutes heating at 63° C., but not at 60° C. (2) that the β principle was rendered inert by heating for 30 minutes at 75° C., but not at 70° C. The inactivation points were thus sharply distinct.

A study was conducted on the action of the 2 principles on their reciprocal, secondary, resistant cultures. When the α principle was permitted to act on the homologous, S type culture secondary colonies, resistant to this principle, arose. Some of these were cultured and, when tested against the β principle, were found to be readily susceptible. When the β principle was permitted to act on the homologous, S type culture secondary, resistant colonies arose in relatively smaller number. These embraced at least 2 types: One, although resistant to the β principle, was susceptible to the α principle. The other, whose mucoid form of growth suggested the intermediate or 0 form of culture, was resistant to both principles.

Cross-tests were performed between the α and β principles of *B. paratyphosus B* and an S type of *B. typhosus*. When the α paratyphoid principle was permitted to act on the typhoid culture on agar plates, no lytic areas appeared. When the β paratyphoid principle was similarly used only small areas in the typhoid culture film developed. When the α and β paratyphoid principles were mixed in a tube and then streaked against the typhoid culture, only the β paratyphoid principle registered; that is, there were only small areas.

A small area (β) typhoid principle, when brought into contact with the original paratyphoid culture on plates, gave only small

areas. The same result was obtained when this typhoid principle was streaked against paratyphoid resistants that had been developed at the expense of the α principle of *B. paratyphosus* acting on the original paratyphoid culture. The β typhoid principle had no influence on paratyphoid resistants that had been developed at the expense of the β paratyphoid principle acting on the original S type of *B. paratyphosus B.*

An attempt was made to prepare antilytic serums for each principle and to study the influence of such serums on the behavior of the respective principles in the course of lysis. Rabbits were immunized and the serums collected. Each serum, in a dilution of 1 :10, was allowed to stand in contact for 2 hours with both principles. The influence of these mixtures on lysis was then observed by the agar plate method. The serum immune to the α principle showed a strong inhibiting influence against this principle when the latter was allowed to act on the original culture. The same serum seemed to have little or no effect on the β principle. The serum immune to the β principle had no effect on lysis by the α principle, and apparently little on lysis by the β principle itself. The last result was unexpected and may have been due to incomplete immunization of the rabbits. This work will be repeated.

The results of these studies, taken as a whole, both confirm and extend certain findings reported earlier by Bail,[1] Bail and Watanabe,[2] Watanabe,[3] Gratia,[4, 5] Hadley,[6] and Kline.[7] They are also in harmony with later observations soon to be reported from this laboratory and involving *B. paratyphosus A.*

Conclusions: From the results reported in this paper, taken in conjunction with those of Bail, Watanabe, Gratia and Kline, it is strongly suggested that the bacteriophage possesses a dual nature. It may exist in the form of either the alpha or the beta units, the former producing the large lytic areas, the latter producing the small. There are no true intermediates, for the variation is discontinuous. While the continued propagation of the alpha principle at the expense of the S type culture results eventually in the generation of the β units, in addition to the α units, the continued propagation of the beta principle, either at the expense of the S type culture or at the expense of the alpha resistants, seems never to determine the generation of the α units. The mode of action of the 2 principles is, in a measure, reciprocal, as pointed out by Gratia[4, 5] for *B. coli.* The alpha principle is active on at least one of the beta resistants; and the beta principle is active on at least one of the alpha resistants. Both principles, on the other hand, act on the

S type culture—which we know commonly possesses a double antigenic configuration. But neither principle acts on certain mucoid resistants arising from the action of the alpha principle on the S type culture.

These observations are impossible of reconciliation with d'Herelle's filtrable virus theory of bacteriophage action, in which the differences in size of the lytic areas (assumed to represent a continuous variation) are correlated with differences in "virulence" of the bacteriophage. And they can be reconciled no more readily with Bordet's theory of nutritive viation. Their significance is still far from clear, but we believe that they offer more substantial support to a conception of the bacteriophage as comprising 2 functionally reciprocal units which may be regarded provisionally as complementary cyclostages in the developmental history of the culture concerned. This conception is developed in greater detail in a series of papers soon to appear.[8]

[1] Bail, O., *Wien. klin. Wchnschr.*, 1922, xxxv, 722.
[2] Bail, O., and Watanabe, T., *Wien. klin. Wchnschr.*, 1922, xxxv, 362.
[3] Watanabe, T., *Zeitschr. f. Immunitätsf.*, 1923, xxxvii, 106.
[4] Gratia, A., *Compt. rend. Soc. biol.*, 1923, lxxxix, 821.
[5] Gratia, A., *Compt. rend. Soc. biol.*, 1923, lxxxix, 824.
[6] Hadley, Ph., *J. Bact.*, 1924, ix, 397.
[7] Kline, G. M., *J. Am. Pub. Health Assn.*, 1927, xii, 1074.
[8] Hadley, Ph., *J. Inf. Dis.*, 1928, (in press).

3855

Anemia Following Splenectomy in White Rats.

PAUL R. CANNON, WILLIAM H. TALIAFERRO AND LESTER R. DRAGSTEDT.

From the Departments of Pathology, Hygiene and Bacteriology, and Surgery of the University of Chicago.

Numerous European workers have observed that rats frequently develop a severe anemia following removal of the spleen. That this is not always the case, however, is shown by the fact that in certain laboratories splenectomy of rats has not been followed by anemia. Lauda[1] made an extensive study of this problem and found that in approximately 75% of his splenectomized rats, a very severe hemolytic type of anemia developed. He proved it to be of an infectious nature and transmissible to other splenectomized rats. Shortly af-

terward, Mayer, Borchardt and Kikuth,[2] in repeating Lauda's work, observed within 24 to 48 hours after splenectomy, small rod-like or dumb-bell shaped inclusions in the erythrocytes. These later increased in numbers until at the height of the anemia there were 12 or more within each erythrocyte, appearing with the Giemsa stain as reddish coccobacillary forms. They concluded that these inclusions were identical with the ones observed by Mayer[3] in 1921 and named *Bartonella muris ratti* because of their similarity to the inclusions found in the erythrocytes of patients with Oroya fever.

We have similar findings in white splenectomized rats. The effect depends primarily upon the source from which the rats were obtained. In 13 rats recently obtained from the Wistar Institute and the Albino Supply Company stock, we have found that no significant anemia follows removal of the spleen. In 2 Wistar rats, however, which had been in the animal room for several months, splenectomy was followed by a very severe anemia. We splenectomized 11 rats obtained from Chicago dealers and in every case, usually about the fifth day, a marked anemia has developed. This anemia appears to be identical with that described by Lauda as "the infectious anemia of rats." The animals show a pronounced pallor of the eyes and mucous membranes, a greatly increased respiratory rate, and in most cases, an intense hemoglobinuria followed by death. The blood picture shows a fall in erythrocytes from 10,000,000 to 2,000,000 per cm., a decrease in hemoglobin from 15.5 gm. to 4-5 gm. per 100 cc. of blood, and a rise in leukocytes from 10,000-15,000 to as high as 70,000 per cm. Giemsa staining reveals a polymorphonuclear leukocytosis. A striking feature at the beginning of the anemia is the marked erythrophagocytosis, many monocytes containing as many as 5 erythrocytes. The erythrocytes contain many *Bartonella* inclusions. A day or so later the blood picture is that of intense blood regeneration with many polychromatophilic cells, noroblasts, reticulocytes and immature leukocytes.

Pathologically, we find the liver enlarged, pale and friable. Sections show the liver cords greatly swollen and the Kupfer cells engorged with erythrocytes in various stages of degeneration. The small intestines contain golden brown fluid and the urinary bladder in most instances contains hemoglobin. The kidneys show a marked nephrosis with iron-containing pigment in the lumina of the convoluted tubules. The bone marrow is actively regenerating with marked erythrophagocytosis in evidence. Sections of the liver, myocardium and kidney stained with scarlet red show distinct changes of the fatty degeneration type.

Etherization or other operations or both might also lead to this anemia. In 8 rats, using such operations as laparotomy and castration or crushing the testicle, removal of one or both adrenals, removal of the frontal lobes of the brain, no significant anemia followed the operation. In 4 of these animals splenectomized from 11 to 13 days after operation severe anemia promptly followed the splenectomy.

In 2 cases thus far we have injected blood from a rat of the infected stock into Wistar rats. Splenectomy of these rats led to the development of a typical fatal anemia in both cases, showing the transmission of the virus.

Our findings further emphasize the great importance of the spleen as a dominant part of the defensive mechanism of the body against latent infections. In addition, we feel that the demonstration of *Bartonella* infections among rats in this country necessitates careful elimination of such infected animals from experiments involving a study of nutrition and particularly from all experiments in which splenectomized animals are used.

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[1] Lauda, E., *Virchows Arch. f. path. Anat.*, 1925, cclviii, 529.

[2] Mayer, M., Borchardt, W., and Kikuth, Walter., *Klin. Wchnschr.*, 1926, v, 559.

[3] Mayer, M., *Arch. f. Schiffs- u. Tropen-Hyg.*, 1921, xxv, 150.

3856

Circulatory Reactions to Ergotamine and Effect Upon Them Produced by Adrenalectomy and the Blood pH.

WILLIAM SALANT, J. ERNEST NADLER AND KEEVE BRODMAN.

From the Department of Physiology and Pharmacology, University of Georgia, and Biological Laboratory, Cold Spring Harbor, L. I.

According to the observations of Rothlin,[1] Dale and Spiro,[2] and Schegg,[3] ergotamine tartrate in doses not exceeding 1.0 mg. per kilo given intravenously to cats and dogs produces a rise of blood pressure. Rothlin reported that very small doses also produced a slight fall of blood pressure in cats, but he ignored this observation in a later communication.[4] His blood pressure tracings showed that the pressor effect was not of exactly the same type. Some showed a persistent elevation of blood pressure while others showed a steep rise followed soon after by a prompt fall. But in all cases there was reversal of action of adrenalin when given soon after the administration of ergotamine.

In reinvestigating the action of this alkaloid we were impressed by the difference in the response of the circulation, under the same experimental conditions. Elevation occurred in some cats and depression was observed in others after the same doses of ergotamine, whatever the anesthetic, whether urethane or ether. The administration of adrenalin given by vein always produced a fall of blood pressure after the first or second doses of ergotamine. After repeated injection of ergotamine, adrenalin usually produced a slight rise of blood pressure, that is, reversal of effect was absent.

The depressor action of ergotamine was very severe and occurred in a large percentage of cases in some groups of experiments. As this bore a striking resemblance to the effect produced by adrenalin given after ergotamine, it occurred to us that the function of the suprarenals might be a causative factor in the production of the depressor action of ergotamine. Removal of both adrenals confirmed our suspicion. In no case did a fall of blood pressure occur after ergotamine, such as was observed with the adrenals intact. There was elevation of blood pressure in more than 50% and no effect in the rest of the experiments. The negative results were probably due to trauma produced by excessive manipulation and exposure of the abdominal viscera in the course of the operation for the removal of the adrenals, which was made by the abdominal route. The fall of blood pressure observed after intravenous injections of ergotamine was apparently caused, therefore, by adrenalin present in the circulation.

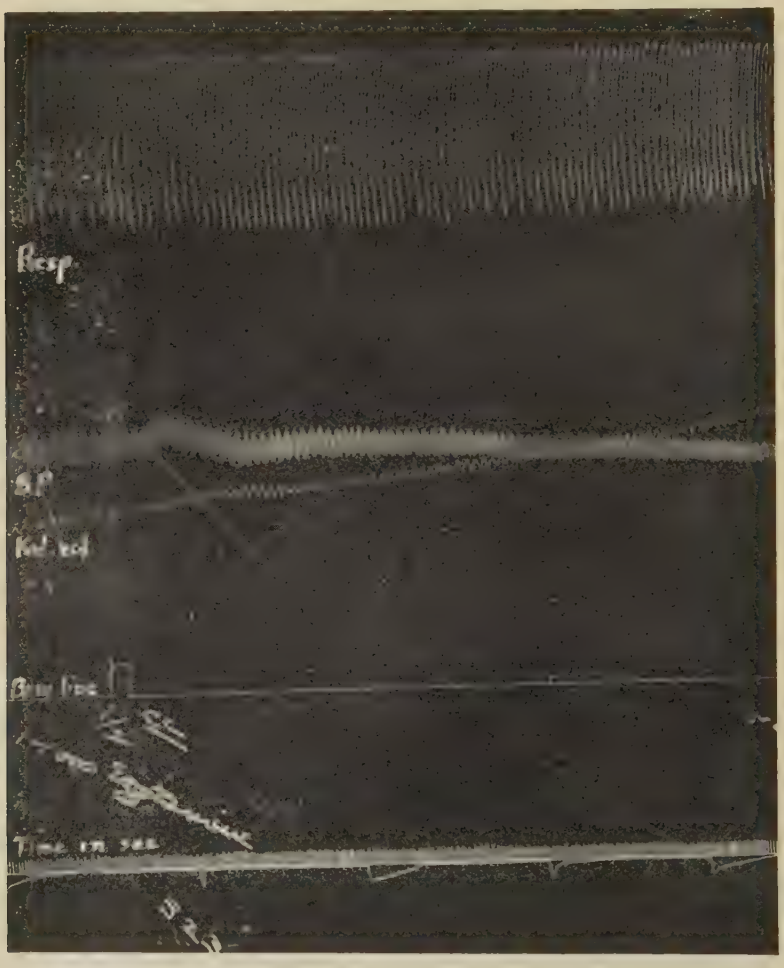
Of interest also is the effect of repetition of the dose. The action was practically uniform whatever the effect of the first injection, a second dose of ergotamine usually producing a slow and persistent rise of blood pressure. Occasionally the third dose would have the same effect as the preceding injection, but sometimes it was less active. If still more of the drug was administered, no change in the circulation was observed, thus corroborating the results obtained by Rothlin in experiments on cats and dogs.

On account of the changes in the reactions of the heart and circulation to drugs produced by alteration in the pH of the blood, as shown by investigations in this laboratory on caffein,[5] cocaine,[6] mercury,[7] the effect of this factor on ergotamine was also studied. Six experiments were carried out on cats, under urethane anesthesia, to which 1% HCl was given intravenously until there was evidence of increased H ion concentration of the blood. The pH of the blood was determined colorimetrically in 4 experiments, and was found to be reduced by 0.1 to 0.2. In 2 experiments in which

the same amounts of acid were given as in the other 4, the pH was not determined, but there was increased respiration and fall of blood pressure with slowing of the heart. In all of these experiments the same effect was produced by the injection of ergotamine, namely, a fall of blood pressure, varying in extent from a few millimeters to about 30 mm. of Hg. When sodium carbonate in sufficient amounts was given intravenously to 6 cats under urethane anesthesia, ergotamine always produced a rise of blood pressure. In one of the experiments this amounted to 15%; in another to 40%; in a third blood pressure rose 33%. In the remaining 3 the increase of blood pressure varied from a few to 9 mm. Hg. (about 10%).

EXP. 14. (C. S. H.) Cat, weight 1.4 kilos. Urethane anesthesia. Seven cc. of 5% sodium carbonate injected intravenously 3 minutes before ergotamine. (Reduced one half.)

In all cases the effect lasted several minutes and was followed by reversal of adrenalin action when this was given after ergotamine, whether the pH was increased or diminished.



EXP. 20. (C. S. H.) Cat, female, weight 2.3 kilos. Urethane anesthesia. 18 cc. 1% HCl injected intravenously shortly before ergotamine. (Reduced one half.)

[1] Rothlin, *Arch. Int. Pharmacol. et Ther.*, 1923, xxii, 459.

[2] Dale and Spiro, *Arch. Exp. Path. and Phar.*, 1922, xcv, 337.

[3] Schegg, *Z. f. Exp. Med.*, 1925, xlv, 368.

[4] Rothlin, *Klin. Wochnschr.*, 4 Jahrg. 30.

[5] Salant, W., and Nadler, J. E., *Am. J. Physiol.*, 1926, lxxviii, 308.

[6] Salant, W., and Nadler, J. E., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 765.

[7] Salant, W., and Nadler, J. E., *J. Lab. and Clin. Med.*, 1927, xiii, 117.

3857

An Error in the Urease Method for the Determination of Urea.*

T. ADDIS.

From the Department of Medicine of Stanford University Medical School.

Estimates of the urea concentration of the blood, muscle and liver tissue of rats were carried out in connection with a study of the effects of high protein diets. The urease method was used. A typical result is given in Table I.

TABLE I.
Urea Concentration in Blood, Muscle and Liver.

Diet	Blood	Muscle	Liver
	mg. per 100 gm.	mg. per 100 gm.	mg. per 100 gm.
Carbohydrate and fat	24	26	207
Protein 74%	52	44	258

The high concentration of urea shown here for the rat's liver is at variance with the observation of Marshall[1] who found an approximate equality of urea concentration in the blood, muscle and liver of other animals. In obtaining the results shown in Table I the rats were anesthetized with ether, and bled to death by cutting the abdominal aorta. The tissues were removed immediately, weighed, ground in a mortar and quantitatively transferred with water to large aeration tubes. The method used has been described in detail elsewhere.[2] The only changes made were those which were necessitated by the fact that the tissues and blood were made up with water to a volume of 30 cc. This method is simply a special adaptation of the aeration method described by Marshall.[1] The urea is calculated from the amount of additional ammonia formed after incubation of the tissue with the filtrate from a suspension in water of freshly ground jack beans.

When the liver was heated before adding the jack bean filtrate an entirely different result was obtained, the previous high level of urea concentration in the liver was no longer found, and the estimates showed an approximate equality of concentration in blood, muscle, and liver, in accordance with the conclusion reached by Marshall. (Table II.)

* This work was aided by the Wellington Gregg Fund for the investigation of Bright's Disease.

TABLE II.
The Effect of Heat on the Urea Concentration of the Liver as determined by the Urease Method.

Liver ground up in boiling water.	Liver ground up in cold water.
mg. per 100 gm.	mg. per 100 gm.
23	296
18	224

A linear relation is attained between the quantities of urea produced and the quantities of "urease" used when the ratio between liver and urease is neither too small nor too large. Fig. 1 shows the result of an experiment in which the liver was held constant at 2.26 gm. and the urease was varied from 0.2 to 1.0 gm. The mixtures were incubated for 2 hours at a temperature of 37° C. The reaction was maintained at pH 7.2 by phosphate.

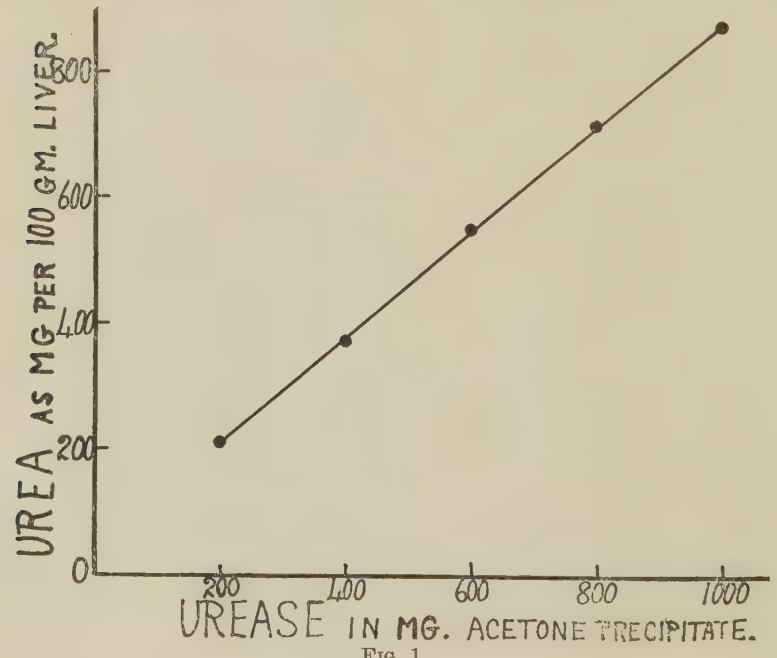


FIG. 1.

In ox blood there is a heat labile agency, confined to the red blood cells, which is capable of forming urea from the acetone precipitate of a water solution of jack beans. The results given in Table III show that the concentration of urea in human blood may be varied by adding different amounts of this acetone treated "urease."

With blood the additional urea produced by increasing the amount of urease is less than when liver is used, but the figures show that the concentration of urea is from 2 to 3 times greater when large

TABLE III.

Increase in Concentration of Urea in Human Blood with increase in amount of Urease added.

Blood and Urease incubated for 60 min. at 38° C.

Subject	Blood	Urease	Concentration of Urea in Blood
	cc.		mg. per 100 cc.
T. A.	2	2 cc. 10% Jack Bean filtrate	33.
"	"	100 mg. acetone precipitate	51.
"	"	500 " "	73.
"	"	1000 " "	77.
L. J. P.	2	2 cc. 10% Jack Bean filtrate	39.
"	"	100 mg. acetone precipitate	67.
"	"	500 " "	120.
"	"	1000 " "	128.
W. S. P.	2	20 mg. acetone precipitate	22.
"	"	200 " "	34.
"	"	400 " "	34.
"	"	800 " "	39.
"	"	1200 " "	40.
E. B.	2	20 mg. acetone precipitate	19.
"	"	200 " "	29.
"	"	400 " "	30.
"	"	800 " "	35.
"	"	1200 " "	35.

than when small quantities of urease are added. This confirms the observations of Miss Behre.[3] Her investigations into the origin of the additional urea led her to the belief that an enzyme present in the soy or jack bean may have formed urea from some unknown constituent of the red blood cells. This hypothesis has not yet been investigated with respect to the blood but it is inapplicable as an explanation of the facts reported here in regard to the liver. For even after "urease" is heated to a temperature at which all known enzymes are inactivated, it still acts as a source of urea formation when incubated with liver. The contrary hypothesis, namely, that the liver contains the enzyme, and that "urease" is the substrate, has therefore been adopted.

No facts have as yet been encountered which would negative the conception that the additional urea is derived from the action of arginase in the liver on arginine in the jack bean. In view of the mode of preparation of some of the bean extracts it is unlikely that this arginine can be free. But proteolytic enzymes in the liver may set arginine free, or it is even conceivable that one of the proteins of the jack bean has arginine so situated in its structure that urea may be formed from it under the action of arginase. An attempt is being made to study these and other related questions.

[1] Marshall and Davis, *J. Biol. Chem.*, 1914, xviii, 52.

[2] Addis, *J. Lab. and Clin. Med.*, 1924, x, 402.

[3] Behre, *J. Biol. Chem.*, 1923, lvi, 395.

3858

The Inhibition of Anaphylactic Shock by the Intravenous Injection of Neoarsphenamine.*

CLAUS W. JUNGEBLUT.

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

We have been particularly interested in the prevention or modification of anaphylactic shock by intravenous injection of colloidal substances into the sensitized animal before reinjection of the antigen, in connection with our studies on the rôle of the reticulo-endothelial system to various immunity phenomena.[1] Extensive studies on the effect of a large number of various colloids (India ink, trypan blue, collargol, casein, etc.), when introduced by the intravenous route into sensitized guinea pigs shortly before reinjection of the antigen, have in our hands, contrary to the findings of other investigators, led uniformly to negative or doubtful results. We have been able, however, to amplify the interesting contributions of Freud[2] and Walbum[3] in a similar field by observing that tuberculous guinea pigs frequently survived a fatal dose of tuberculin when this was preceded (a few hours) by an intravenous dose of India ink. More significant results, of which the following is a brief report, have recently been obtained with the intravenous administration of neoarsphenamine in sensitized guinea pigs shortly before reinjection of the specific antigen.

In one experiment 20 guinea pigs, weighing from 250 to 350 gm., were sensitized by the subcutaneous injection of 0.01 cc. of normal horse serum. The minimum dose of antigen, capable of producing after intravenous injection death from acute anaphylactic shock within 3 to 5 minutes, was determined in 4 animals, 3 weeks later, and found to be 0.3 cc. The remaining 16 animals, in groups of 4, were given at the same time, 1 cc. of a 1:50 dilution of neoarsphenamine intravenously, preceding the intravenous reinjection with 0.3 cc. of normal horse serum at intervals of 5 minutes, 15 minutes, 30 minutes and one hour. The 4 animals which had received the antigen 5 minutes after the injection of the arsenical showed no immediate symptoms, but died from protracted shock within 20 to 35 minutes. Of those injected after the 15 minute interval, 2 survived

* The experiments reported in this paper were carried out in part at the Department of Bacteriology of Columbia University, New York City, while holding a fellowship in medicine of the National Research Council.

without exhibiting more than transitory dyspnea and slight anaphylactoid symptoms such as were seen in normal guinea pigs from the dose of neoarsphenamine employed; the other 2 died with ill-defined symptoms within 10 to 20 minutes. With the 30 minute interval, one animal survived and 3 died more suddenly, while in the last group, which had received the neoarsphenamine one hour previously, death occurred in all animals with typical anaphylactic shock within 3 to 5 minutes. With the exception of the last group, autopsy of the dead animals did not reveal the characteristic inflation of the lungs. The 3 surviving animals of this series were reinjected intravenously 24 hours later with 0.5 cc. of normal horse serum in order to determine the presence of anti-anaphylaxis. All survived without exhibiting any symptoms whatsoever.

In another experiment 12 guinea pigs of the same weight were sensitized passively by the intraperitoneal injection of 1 cc. of an anti-horse rabbit immune serum, which gave a marked precipitin reaction with the antigen up to 1:2000 dilution. The minimum dose of horse serum, producing actual fatal shock after intravenous injection, was determined 24 hours later in 4 animals and found to be 0.05 cc. The remaining 8 animals, in groups of 4, were given at the same time intravenously 1 cc. of a 1:50 dilution of neoarsphenamine 15 and 30 minutes, respectively, before the intravenous injection of 0.05 cc. of normal horse serum. Of the 4 animals receiving the antigen 15 minutes after the drug, 3 survived, showing only slight initial dyspnea, one died within 25 minutes in protracted shock; of the 4 injected after the 30 minutes interval, 2 survived and 2 died within 10 to 20 minutes. The surviving animals were tested the following day for antianaphylaxis and were found to be fully protected against 0.5 cc. of normal horse serum.

Although the number of animals in these 2 experiments is too small to draw definite conclusions, it appears that the intravenous injection of neoarsphenamine into actively and passively sensitized guinea pigs, if given 15 to 30 minutes before the introduction of the specific antigen, was capable of saving at least 50% of the animals, while the rest died during prolonged prostration from a modified shock which did not include the classical symptoms of acute anaphylaxis. It is difficult to determine accurately at present the mechanism of this inhibitory action. The fact that the surviving animals were antianaphylactic 24 hours later points to a specific desensitization which makes an explanation based solely on a temporary disturbance of the blood colloids somewhat unlikely. The production of an effect analogous to a blockade of the histiocytes, for which the drug undoubtedly has a strong affinity,[4] or an action

on other susceptible tissue cells, seems to be highly improbable since the protective effect of the neoarsphenamine appeared to be definitely limited to the time during which the drug is present in the circulation at the maximal concentration. An adsorption of complement cannot be considered more than possibly a contributing factor inasmuch as we found that even as strong an adsorbent as yeast cells, when introduced intravenously into a sensitized guinea pig, did not protect against a subsequent shocking injection, but rather, on the contrary, provoked in itself in some cases very marked symptoms resembling true anaphylactic shock. In view of the pronounced anticoagulant properties of the arsphenamines, one might be tempted to assume such an action as the cause for the observed protection, were it not for the fact that recent studies by Hanzlik, Butt and Stockton,[5] Reed and Lamson[6] and Hyde[7] have apparently refuted the earlier claims of Kyes and Strauser[8] and Williams and van de Carr[9] on the shock-preventing action of heparin. Finally, one might think of a denaturizing effect of the arsenical on the antigen which would entail a change of the immunological specificity of the latter, since the injection of an *in vitro* prepared mixture of antigen and neoarsphenamine was likewise tolerated by some sensitized animals, without being followed by death from anaphylactic shock. Such an hypothesis would seem to be supported by the earlier work of Swift[10] and Landsteiner,[11] and would find an interesting analogy in the more recent studies of Steppuhn, Zeiss and Brychonenko,[12] Schmidt,[13] Makarowa and Zeiss,[14] and Iwanoff[15] on the shock preventing and biological properties of "Germanin" (Bayer 205).

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[1] Jungeblut, C. W., and Berlot, J. A., *J. of Exp. Med.*, 1926, xliv, 129.

[2] Freud, J., *J. of Immun.*, 1926, xi, 383.

[3] Walbum, L. E., *Compt. rend. Soc. biol.*, 1926, xciv, 1106.

[4] Jungeblut, C. W., *Z. f. Hyg. u. Inf. Kr.*, 1927, cvii, 357.

[5] Hanzlik, P. J., Butt, E. M., and Stockton, A. B., *J. of Immun.*, 1927, xiii, 409.

[6] Reed, C. I., and Lamson, R. W., *J. of Immun.*, 1927, xiii, 433.

[7] Hyde, R., *Am. J. of Hyg.*, 1927, vii, 614.

[8] Kyes, P., and Strauser, E. R., *J. of Immun.*, 1926, xii, 419.

[9] Williams, O. B., and van de Carr, F. R., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 798.

[10] Swift, *J. Am. Med. Assn.*, 1912, lix, 1236.

[11] Landsteiner, K., *J. Exp. Med.*, 1924, xxxix, 631.

[12] Steppuhn, O., Zeiss, H., and Brychonenko, S., *Arch. f. Schiffs. Trop. Hyg.*, 1923, xxvii, 206.

[13] Schmidt, H., *Z. f. Imm. Forschg.*, 1926, xlv, 496.

[14] Makarowa, J., and Zeiss, H., *Z. f. Imm. Forschg.*, 1926, xlvii, 110.

[15] Iwanoff, K., *Z. f. Hyg. u. Inf. Kr.*, 1927, cviii, 152.

3859

Observations on Mitochondrial Growth in Artificial Culture Media.

IVAN E. WALLIN.

From the Department of Anatomy, University of Colorado School of Medicine.

In former publications,[1] it was shown that when rabbit liver from new-born rabbits is planted in rabbit liver infusion media containing urea (0.5%), the mitochondria of the planted livers will grow independently in the culture medium. Two types of growth develop in the media: surface growth, which is similar to ordinary bacterial surface growth, and a deep growth which produces a clouding in the medium round about the planted liver tissue. It was shown that the clouded growth in some instances could be subcultured to produce a surface growth.

In recent experiments it has been again very evident that the deep clouded growth is a true bacterial growth. This is shown by the fact that when surface growths have developed on the media there has been no, or very little, deep growth. So also, when the deep clouded growth developed there has been no surface growth. In a few instances where neither surface nor deep growth developed in the usual time, it was found that surface growth may ultimately appear; in one case, 21 days after the tissue was planted. Thus, it may be stated that independent mitochondrial growth occurs in practically 100% of cases when liver of new-born rabbits is planted in the media.

Considerable time has been spent in attempting to determine the factors responsible for surface growth in some instances and the deep clouded growth in other cases. So far, I have been able to recognize certain general conditions only. The significance of the various ingredients of the culture media have been studied, but any pronounced significant factors have not been recognized. Water used in the media was distilled in various ways and obtained from different sources. There appeared to be some variation in the results from the different waters used, but the results were not constant. Another ingredient of the media, namely peptone, is difficult to evaluate. Witte's peptone has been used in the preparation of all media. To what extent the heat in sterilization produces changes in this complex ingredient is not known, but it appears probable that heat produces changes in the peptone and that these changes will not be the same in the preparation of different batches of media.

While the chemical constitution of the medium appears to be the

prime factor in the successful culture medium, the physical condition of the medium also appears to play an important rôle. The consistency of a medium may vary from a very firm jelly to one that is barely solid depending on the amount of water present. In many instances it appears that a particular consistency is favorable to the production of surface growth.

It has become evident that the mitochondria vary in their physiology in different new-born rabbit livers, and that the physiological state is an important factor in determining the character of growth in the culture media. A variation in the response of mitochondria is shown in stained smear preparations of liver from different new-born rabbits. In some cases the mitochondria are well preserved and numerous, in other cases they are few in number. The gross characteristics of the liver also vary in the new-born rabbits. The color varies from a yellowish brown to a deep maroon. So also, the physical nature of the livers vary; some are stringy and tough, while others are friable and easy to cut into pieces.

Mitochondria appear to be extremely delicate in their responses. With variable factors in the culture media on the one hand and variable physiology of the mitochondria on the other, it appears nigh impossible to devise a culture medium that will constantly produce surface growth of mitochondria.

[1] Wallin, Ivan E., *Am. J. Anat.*, xxxiii, 1; xxxv, 3; xxxvi, 1.

3860

Experimental Typhoid Fever Induced in Guinea Pig With In Vivo Prepared B. Typhosus Toxic Product.*

WILLIAM E. HARRIS.

From the Department of Pathology, Tulane University, New Orleans, La.

Since typhoid fever represents a syndrome manifestly accompanied by toxemia, many and varied attempts have been made to obtain the specific toxic moiety from the typhoid bacillus. While certain of these procedures have yielded toxic materials which when injected into animals produce tissue reactions, they are not, as far as ascertained, analogous to the pathological changes occurring in typhoid fever of man.

* Aided by a grant from the David Trautman Schwartz Research Fund.

The toxic material employed in the experiments herein reported was prepared through an *in vivo* method, *i. e.*, the activities of the living animal body were used to produce in part or set free a toxic

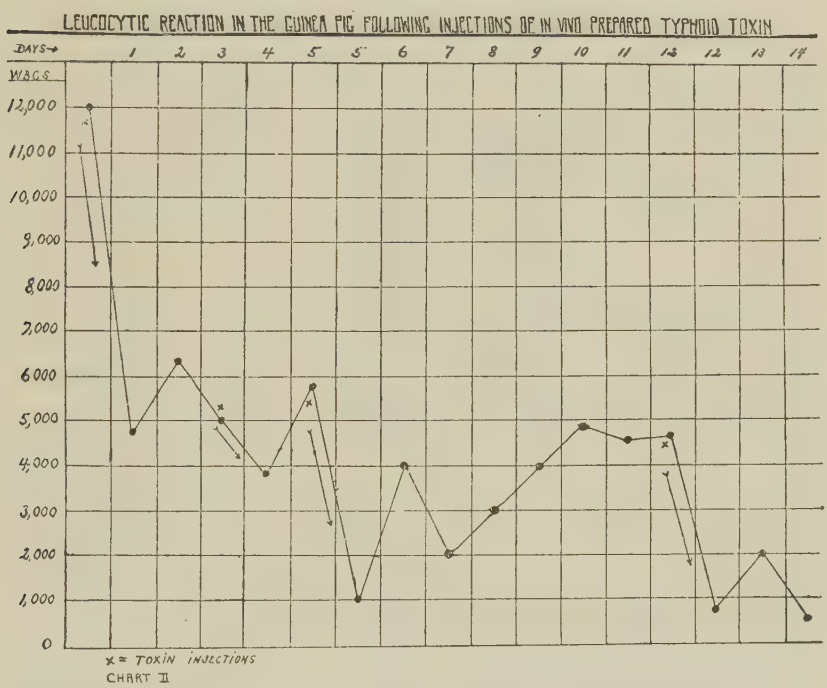

CHART II

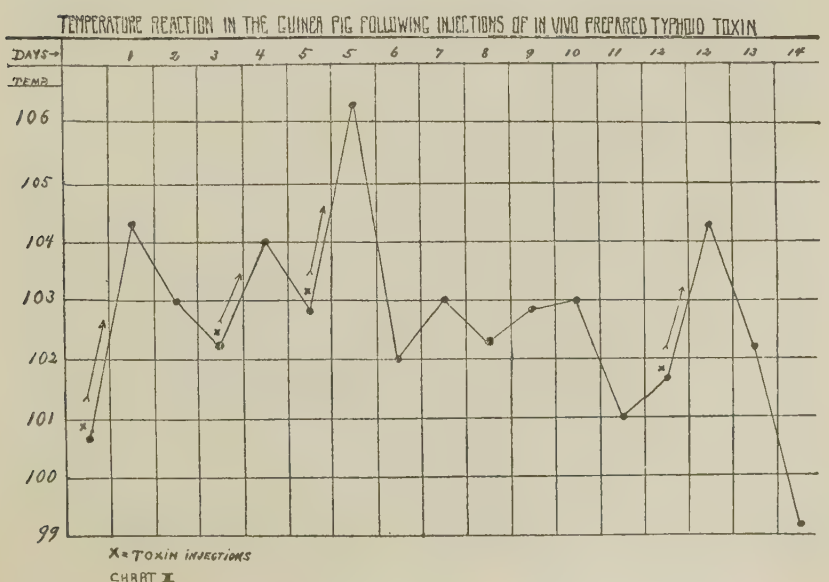

CHART I

portion of the microorganism. By this method peritonitis was first produced in guinea pigs with cultures of *B. typhosus* and the animals were killed after they had become sick, which was usually within 8 to 12 hours. After coarse filtration this exudate was filtered through a Berkefeld letter N filter. A series of guinea pigs were injected with the filtrate thus prepared, by different routes, some subcutaneously, some intracardially and others intraperitoneally. Several injections were given to each animal of a series at intervals of from 2 to 4 days. Subsequent to each injection, irrespective of the route, a febrile rise and marked leucopenia occurred. (See charts.) Death of the animals usually occurred in from 2 to 4 weeks.

At autopsy the chief gross pathological changes were noted in the intestine, lymph nodes, spleen, liver and kidney. The peritoneal lymph nodes were markedly enlarged and at times hemorrhagic. Peyer's patches and the solitary follicles of the intestinal tract were much swollen and elevated; in some instances they were somewhat reddened and revealed early ulceration. The spleen was increased in size, soft and congested. The liver was congested and often showed small scattered yellowish areas of what appeared to be necrosis. The kidneys, adrenals and bone marrow were swollen and congested. The lungs and heart appeared normal and it is noteworthy that in no instance was pneumonia present.

The microscopic study of the lymphoid structures, especially of the peritoneal cavity, including Peyer's patches and the solitary follicles of the intestinal tract, showed the enlargement to be due to an apparent hyperplasia of the lymphoid elements and also to the presence of multiple large, pale and more or less oval cells, many of which were phagocytic and correspond to the type emphasized by Mallory[1] as the principal reactionary cell of human typhoid. These phagocytes had engulfed lymphoid cells and nuclear fragments and at times red blood cells, depending upon the area of their location. In some of the nodes, areas of marked congestion and hemorrhage and small foci of necrosis were present. In the Peyer's patches of certain of the animals, necrosis, loss of the epithelial lining, and rupture of the muscularis mucosa were noted, *i. e.,* early ulceration had occurred. The splenic pulp was markedly congested and in some areas hemorrhagic extravasations were observed. Many of the erythrocytes showed a shadow-cell type due apparently to the loss of hemoglobin. Numbers of "endothelial" phagocytic cells were seen which contained "shadow red-cells" within their cytoplasm. The liver demonstrated focal necrosis of varying extent.

In some areas complete necrosis of the cells had occurred and altered red blood cells were present, many of which were engulfed by the phagocytic cells. In the smaller areas of focal necrosis, the liver cells were replaced by "endothelial" or phagocytic cells. One outstanding feature in the microscopic study of the tissues of all animals, was the absence of reactionary polymorphonuclear neutrophiles.

FIG. 1.

Portion of small intestine of the guinea pig (experimental typhoid fever) showing the type of gross lesion produced in Peyer's patch.

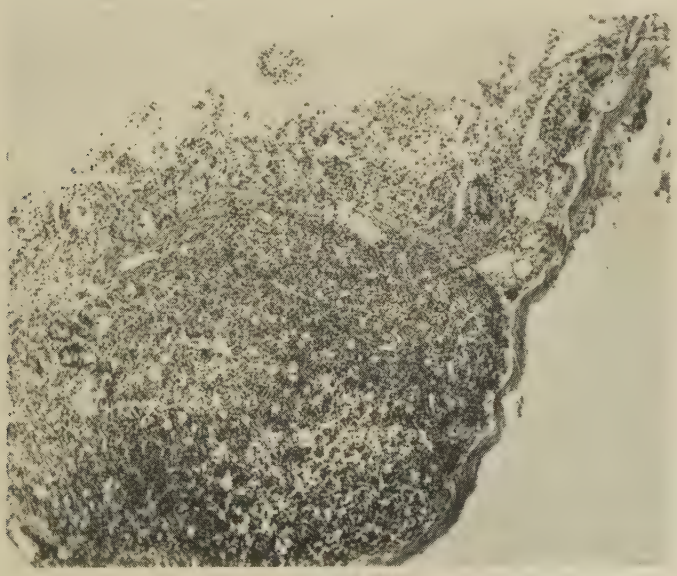

FIG. 2.

Low power photomicrograph of a portion of Peyer's patch of guinea pig, showing marked enlargement. There are many phagocytic "endothelial" cells and a hyperplasia of lymphoid elements. The muscularis mucosa is in part lost, and the overlying mucosa destroyed.

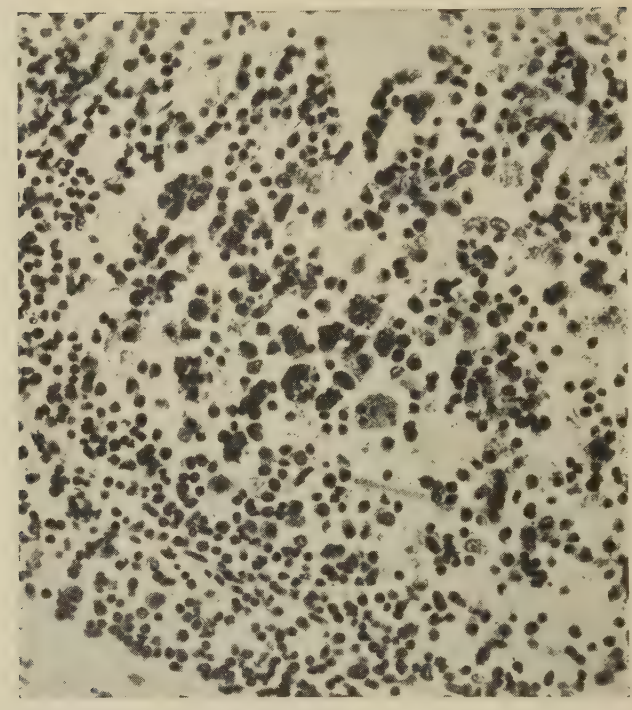

FIG. 3.

High power area of Peyer's patch shown in Figure 2, demonstrating phagocytes containing numbers of lymphoid cells and nuclear fragments.

It would appear from this study that when a peritonitis is produced in the guinea pig by *B. typhosus,* a toxic substance is liberated during the encounter between the invading microorganisms and the host which is very analogous if not identical in its action with that of the toxin present in typhoid infection of man. Whether the toxic moiety is secreted by the microorganism during its invasion of the somatic host structures, or whether it is discharged through the injury of the bacilli by the body tissues and fluids, or is produced through both such activities, has not been determined.

The results obtained in these experiments indicate that through the employment of this *in vivo* method, a bacterial free filterable toxic substance can be produced from the typhoid bacillus which when injected into the guinea pig produces effects and tissue lesions analogous to those occurring in human typhoid infection.

[1] Mallory, F. B., *J. Exp. Med.*, 1898, iii, 611.

3861

The Effect of Freezing on Yeasts.

F. W. TANNER AND BEATRICE W. WILLIAMSON.

From the Department of Bacteriology, University of Illinois, Urbana.

The effect of freezing on yeasts has not received much study, at least under conditions which give information concerning their longevity, rate of death, etc. Doemens[1] exposed beer yeast to a temperature of about—190° C. for six minutes. It still retained its vitality. When he suspended the yeast in water and exposed to liquid air for 5 minutes and 20 minutes, and then thawed in cold water, its power of development was totally destroyed. Macfadyen and Rowland[2] subjected microorganisms, among which were yeasts, to a temperature of —252° C. for 6 months, after which the yeasts were reported to have suffered no reduction in vitality. They gave good growth and possessed unaltered powers of fermentation. One would infer from this report that yeasts are resistant to freezing and that there was no reduction in numbers. Bokorny[3] kept a sample of brewers' pressed yeast at a temperature of —15° C. for 24 hours and then allowed it to warm up slowly to a temperature of 7° C.; fermentation and reproduction went on for a short time but soon stopped. In another experiment the yeast was very quickly raised to 10° C.; after 4 weeks in a suitable medium it exhibited slight fermentation. Microscopic examination at this stage showed that most of the cells were dead and only a few were budding. Bokorny thus showed that beer yeast was resistant to cold but not as resistant as other microorganisms.

In the experimental work reported here 8 species of yeasts were used; 4 species of common bacteria were also included in order to act as controls since so much work has been done on the effect of freezing bacteria. *Escherichia coli, Serratia marcescens, Bacillus mesentericus* and *Bacillus subtilis, Saccharomyces cerevisiae, Saccharomyces ellipsoideus, Saccharomyces pastorianus, Saccharomyces marxianus, Pichia membranaefaciens, Zygosaccharomyces mongolicus, Torula rosea,* and *Mycoderma vini* were used. These organisms grew well on the ordinary laboratory media; the bacteria on plain media and the yeasts on glucose or malt media. The procedure for each of the organisms was as follows: 2 cc. of a 24 hour broth culture of the organism were transferred by means of a sterile syringe to each of 60 sterile vaccine ampoules which were then sealed immediately with a Bunsen flame. In like manner 60

TABLE I.

	Escherichia coli		Serratia marcescens		Bacillus mesentericus		Bacillus subtilis	
	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension
Initial count	18,500,000	212,000,000	24,000,000	145,200,000	2,100,000	17,600,000	1,850,000	15,000,000
Frozen 3 wks.			2,100,000	120,000,000				
" 4 wks.	2,000,000	6,000,000			1,200,000	15,000,000	1,200,000	9,500,000
" 5 wks.	1,200,000	4,650,000					1,500,000	7,000,000
" 6 wks.	50,000	3,000,000	100,000	110,000,000	1,100,000	13,200,000		
" 8 wks.	16,500	2,950,000	36,000	50,000,000	800,000	5,800,000	100,000	5,400,000
" 9 wks.	1,400	1,100,000	18,000	42,000,000	950,000	10,000,000		
" 10 wks.	300	1,230,000			600,000	7,000,000		
" 12 wks.	1,400	990,000	10,000	3,000,000	650,000	7,000,000	23,000	5,200,000
" 16 wks.	100	20,000	7,000	90,000	430,000	6,500,000		
" 18 wks.	40	18,000			500,000	6,000,000	13,000	5,100,000
" 20 wks.	1	15,000						
" 21 wks.	sterile	5,000						
" 22 wks.	sterile							
" 32 wks.			5,000	12,000				
" 34 wks.					250,000	4,000,000	14,000	4,500,000
" 38 wks.	sterile	sterile	100	10,000	130,000	3,400,000	11,000	5,100,000
" 80 wks.			360	100	23,000	2,500,000	2,000	200,000

TABLE II.

	Saccharomyces cerevisiae		Saccharomyces ellipsoideus		Saccharomyces pastorianus		Saccharomyces marxianus	
	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension
Initial count	300,000	6,900,000	1,050,000	3,750,000	1,600,000	4,000,000	30,000	3,500,000
Frozen 2 wks.					100,000	900,000	16,000	150,000
" 4 wks.	15,000	4,700,000	700,000	1,300,000	80,000	50,000		
" 5 wks.	10,300	4,400,000	1,200	170,000			7,000	39,000
" 6 wks.					55,000	64,000		
" 7 wks.	10,000	3,120,000	34	19,000	50,000	53,000	4,200	19,000
" 8 wks.			20	10,000	38,000	42,500		
" 9 wks.	8,800	2,000,000	17	11,500				
" 10 wks.	5,500	1,900,000	16	10,000	40,000	38,000	5,300	6,400
" 12 wks.	7,000	400,000	10	12,000	18,000	40,000	6,000	7,000
" 14 wks.	6,500	190,000	6		10,000	30,000	4,500	3,800
" 16 wks.			4	8,000			3,000	4,000
" 18 wks.			7	3,000				
" 22 wks.			5	600				
" 30 wks.	3,000	32,000			6,500	30,000	1,700	3,400
" 38 wks.			1	400				
" 58 wks.	2,000	64,000	1	40	1,300	30,000	300	1,000
" 66 wks.	2,300	60,000	3	200	3,000	30,000	600	5,000
" 80 wks.	1,500	65,000	0	250	2,400	21,000	400	4,000
" 160 wks.	950	53,000	0	95	1,600	19,000	120	2,200

TABLE III.

	Pichia membranaefaciens		Torula rosea		Zygosaccharomyces mongolicus		Mycoderma vini	
	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension
Initial count	70,000	3,300,000	4,300,000	5,000,000	24,000,000	2,000,000	1,800,000	48,000,000
Frozen 2 wks.	20,000	200,000	100,000	3,300,000			1,300,000	38,000,000
" 4 wks.	1,000	50,000	60,000	700,000	3,000,000	800,000		
" 5 wks.	900	11,000	10,000	290,000			1,000,000	41,000,000
" 7 wks.	13,000	8,000	7,000	130,000	800,000	950,000	850,000	27,000,000
" 9 wks.	500	1,000	5,000	20,000	100,000	130,000	600,000	18,000,000
" 10 wks.			3,000	20,000	150,000	200,000		
" 26 wks.	sterile	sterile	160	1,500	10,000	60,000	150,000	5,000,000
" 46 wks.	sterile	sterile	1	500	200	20,000	8,000	260,000
" 54 wks.	sterile	sterile	2	2	500	1,000	500	110,000
" 68 wks.	sterile	sterile	3	1	200	800	100	60,000
" 160 wks.			sterile	sterile	3	2	20	2,000

sterile ampoules were filled with 2 cc. of a suspension of these organisms in physiological NaCl solution. These suspensions were prepared from 24 hour agar slant cultures and shaken thoroughly on a shaking machine to insure an even suspension. Plate counts were made of the broth culture and of the suspension to determine the initial count before freezing. The ampoules were then placed in the trays of an electric refrigerator of the Frigidaire type at a temperature ranging from —13° to —15° C. This temperature was maintained constantly throughout the experiment except for one day when the temperature was a little above zero. The ampoules were removed at regular intervals, allowed to melt at room temperature, shaken thoroughly, and 1 cc. of the contents plated in different dilutions to determine the change in the count if any had occurred.

The accompanying tables summarize the results of the experiment, giving the initial counts of the broth culture and of the suspensions, and the counts obtained after the cultures have been frozen for various lengths of time. Table I is a record of the bacterial counts; Tables II and III are records of yeast counts.

These data indicate that the number of living cells in suspensions of bacteria and yeasts undergoes a rapid reduction at first; after a longer period of time the reduction is very slow. Non-spore-forming bacteria were much more susceptible to the harmful effects of freezing than the spore-forming species. A great variation in resistance to temperatures below freezing was noticed among the yeasts which were used.

Conclusions: 1. The prolonged action of freezing temperatures destroys the cells of yeasts and bacteria. 2. The rate of death follows the curve of a monomolecular reaction, the death rate being proportional to the number of living cells. 3. There is a great variation among yeasts and bacteria in their resistance to freezing. 4. Under the conditions of this experiment, the death rate seemed to be slower in the physiological sodium chloride solution than in the broth.

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[1] Doemans, reviewed in *J. Fed. Inst. Brew.*, 1901, vii, 299.

[2] Macfadyen, A., and Rowland, S., *Ann. Bot.*, 1902, xvi, 589.

[3] Bokorny, M., *Brau. u. Hopfen-Zeit.*, 1927, cxli; reviewed in *J. Inst. Brew.*, 1927, xxxiii, 520.

3862

Creatine Excretion in Artificial Hyperthyroidism.

DONALD A. CARSON. (Introduced by Arthur L. Bloomfield.)

From the Department of Medicine, Stanford University Medical School.

An attempt was made to produce creatinuria in 3 individuals by the ingestion of thyroid extract. Palmer[1] has recently reported that creatine is excreted in the urine of patients suffering from hyperthyroidism and that under iodine medication this excretion tends to disappear. Creatine determinations were made by the method of Folin.[2] Tests for acetone and diacetic acid in the urine were negative. The first subject, an arterio-sclerotic individual of 63, having an initial basal metabolic rate of —22, was placed on a meat-free diet with 1.25 gm. of protein per kilogram of body weight. After observing his creatinine excretion he received gr. 19 of thyroid extract (Armour) over a 6-day period, then gr. 6 daily over a 4-day period, then gr. 9 daily for 8 days, and finally gr. 6 daily for 16 days. Creatine appeared in the urine on the second day of thyroid administration, disappeared on the twelfth, and then rose to a maximum excretion of 483 mg. on the twentieth day of the experiment. This coincided with a B.M.R. of +33. When the dose of thyroid was decreased to gr. 6 daily and 1 cc. of Lugol's solution was given twice a day for 7 days the creatine excretion fell to 100-

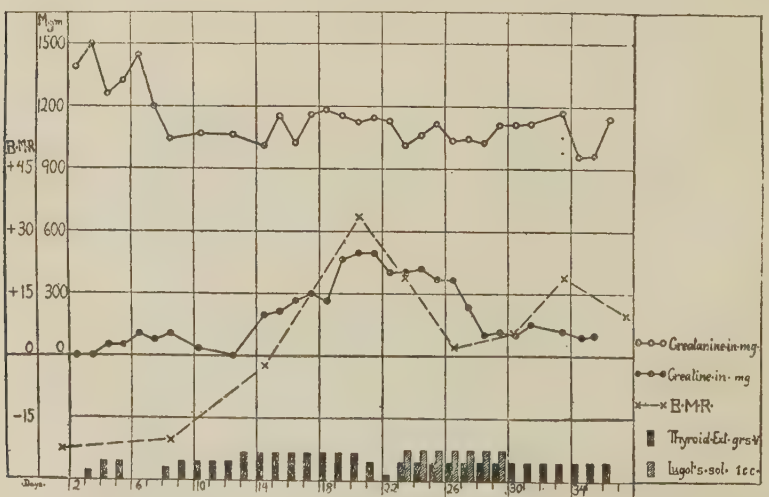

CHART. Effects of Thyroid Extract and Lugol's Solution on Excretion of Creatinine and Creatine in Urine. The blocked and hatched areas in the abscissa represent doses of thyroid and of Lugol's solution.

150 mg. a day. This was not an iodine effect, however, as continued administration of thyroid without iodine was not sufficient to maintain metabolism at its previous high levels or to increase creatine excretion. The average daily excretion of creatinine amounted to 1350 mg. During the experiment this fell to a level averaging 1050-1100 mg. The complete figures are shown in the accompanying chart.

The second subject, a normal male of 29, having an initial B.M.R. of —5, on an unrestricted diet, took daily doses of thyroid extract (Burroughs and Wellcome) increasing from gr. 10 to gr. 35. Urine collections were irregular but there was a creatine excretion of 36-221 mg. per day in the earlier part of the experiment without significant changes in the excretion of creatinine. Observations were discontinued before the B.M.R. was materially elevated.

The third subject was a normal male of 31. The initial B.M.R. was +4. He was placed on a diet similar to that given subject 1, and took thyroid extract (Armour) in daily doses of gr. 5 for 6 days, gr. 6 for 1 day, gr. 9 for 3 days, and gr. 12 for 10 days. The creatinine excretion was constant during 18 days. Creatine appeared in the urine on the third day of medication, disappeared on the sixth day, reached a maximum of 178 mg. on the eighth day, and finally disappeared on the fifteenth day, not to reappear during the next three days. The B.M.R. was at its height, +23, at this time. The effect of iodine medication on creatinuria could not, therefore, be observed.

Creatinuria, then, can be produced in the human by the ingestion of thyroid extract but it can not be maintained from day to day unless a severe intoxication be developed. The disappearance of creatine from the urine in the earlier part of the experiment in subjects 1 and 3, and its complete disappearance in the case of subject 3 when the basal metabolism was at its height is an unexplained observation which should be investigated. As there was no correlation between the dose of thyroid and the creatine excretion it was probably not due to the presence of creatine or its precursors in the thyroid extract. It suggests that thyroid substance may have an effect on protein metabolism separate and apart from its effect on metabolism in general.

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[1] Palmer, W. W., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 229.

[2] Folin, O., *J. Biol. Chem.*, 1914, xvii, 469.

3863

Weight, Body Diameters and Age; Correlation Coefficients.*

H. GRAY. (Introduced by Herman M. Adler.)

This is an attempt to improve the height-age style of weight prediction table currently used both for adults and for children. The measurements studied are: weight, net (W), age (A), stature (S), bi-cristal diameter of pelvic brim (Bc), bi-styloid diameter of wrist (Bs), bi-malleolar diameter of ankle (Bm). The material consists of private school boys aged 4 to 20 years, to the number of 810, except for the diameters of wrist and ankle with populations of 290 and 293 respectively. The linearity of some of the distribution plots is open to question. For instance, weight on stature looks curvilinear at the extremes and the criterion $n^2—r^2 = .0229 \pm .0072$. Nevertheless, I am told by other people familiar with statistics that the plots are sufficiently rectilinear for the present tentative analysis.

The resulting correlation coefficients may be examined in Table I. If other things be equal, that trait most highly correlated with weight would be expected to be the most dependable for predicting weight. If we note especially the relationship of weight to various traits, it appears that this is highest for stature, then bi-cristal, then bi-styloid, then age, and finally bi-malleolar. Furthermore the difference between r_{wA} and r_{ws} proves to be statistically significant; $.0379 \pm .0036$, yielding a ratio of the difference to its probable error of 10.4. Likewise the difference between r_{wA} and r_{wBc} is statistically significant: $.0240 \pm .0045$, ratio 5.3. But r of weight with bi-styloid diameter of the wrist, although larger than r_{wA}, is not significant: $.0047 \pm .0070$. Hence one may conclude that weight is best referred to stature, next best to bi-cristal diameter, only third best to age, while wrist diameter is not worth further consideration.

TABLE I.

Correlation coefficients, Zero Order, in Order of Magnitude.

Stature:Age	.9320 ± .0027	Weight:Bi-styloid	.8936 ± .0080
Weight:Stature	.9268 ± .0036	Weight:Age	.8889 ± .0054
Bi-cristal:Stature	.9234 ± .0035	Bi-cristal:Age	.8700 ± .0058
Weight:Bi-cristal	.9129 ± .0041	Weight:Bi-malleolar	.8606 ± .0103
Bi-styloid:Stature	.9028 ± .0074	Bi-styloid:Age	.8576 ± .0103
Bi-malleolar:Stature	.8971 ± .0076	Bi-malleolar:Age	.8334 ± .0124

* This is one of a series of articles by members of the staff of the Behavior Research Fund, Illinois Institute for Juvenile Research, Chicago, Herman M. Adler, M.D., director. Series B, number 120.

Other things, however, are not equal, and therefore partial correlation coefficients are desirable in order to render constant those variables which are likely to affect the straight correlation. The first order partials show that, when age is fixed, weight is slightly more highly correlated with bi-cristal diameter ($r = .61$) than with stature ($r = .59$), whereas when stature is fixed weight is more highly correlated with *bi-cristal* diameter (.39) than with age (.18).

The second order partials show that weight is distinctly more highly correlated with bi-cristal when stature and age are fixed ($r = .39$) than with age when stature and bi-cristal are constant ($r = .27$). Hence it may be expected that the multiple correlation of weight with stature and bi-cristal will be better than with stature and age.

The multiple correlation coefficient R of weight on various pairs of traits, each pair containing stature, follows:

$R_{w(SBc)} = .9435 \pm .0026$ $\quad$ $R_{w(SBm)} = .9292 \pm .0054$

$R_{w(SBs)} = .9362 \pm .0049$ $\quad$ $R_{w(SA)} = .9294 \pm .0032$

Furthermore the difference between the first and last of these R's is statistically significant. $.0141 \pm .0015$, ratio 9.5. The difference between the second and last R's $= .0068 \pm .0027$, yielding the ratio Diff./P.E. of Diff. $= 2.5$; this ratio should be 3 or more to be regarded as significant, and perhaps it might be that large if the number of Bistyloid measurements had been, instead of 290, as large as the number of Bi-cristal diameters, namely 810; in sum it seems more profitable to omit the wrist diameter for the present and to concentrate attention on the breadth of the pelvic brim (Bc).

The main inference seems that weight is less closely related to age than to certain body diameters, which therefore merit further study with a view to substitution for age in tables for prediction of normal weight.

3864

Relation of Salt Deficient Diets to Resistance to Infection.

I. J. KLIGLER AND A. GEIGER.

From the Department of Hygiene, Hebrew University, Jerusalem.

Recent experiments, notably those reported by Webster and Pritchett,[1] have shown that diet has a decided influence on susceptibility to infection. These investigations dealt with the vitamine fraction of the diet. It seemed to us of importance to ascertain the part

played by salts in the metabolic process and in host resistance to disease.

Rats were used as experimental animals and trypanosomes as the infectious agent. Rats from the same stock were kept on a synthetic diet* from which one or another cation was eliminated. After a certain period equal numbers of rats of approximately the same age or weight were infected with *Tr. evansi,* each rat receiving the same number of trypanosomes intraperitoneally. The duration of the infection was used as an index of individual resistance.

The results indicate a decreased resistance in the rats on a salt deficient diet as compared with those maintained on a full or standard diet. A summary of the results obtained is shown in Table I.

TABLE I.

Series	No. of Rats	Salt Deficient	Average Weight	Dose	Average Survival Time
1.	4	K free	26-29	2,500	18 days
	4	Mg. free	" "	"	16 "
	4	Standard	" "	"	22 "
2.	3	Na† free	23.5	6,000	17 "
	4	Mg. free	36	6,000	20 "
	3	Standard	39	6,000	24 "
3.	6	Mg. poor	58	10,000	18.3 "
	6	Mg. rich	67	"	18.0 "
	4	Standard	51	"	21.0 "
4.‡	2	Ca free	26.5	5,000	26 "
	3	Na free	27.0	"	19 2/3 days

†The Na rats were of about the same age as the others but much smaller in weight.

‡Second generation.

It is evident that elimination of any of the important salts causes a reduction in resistance, the average survival time being 20-30% less than on a full diet. The rats fed diets poor in salt remain dwarfed, with the exception of Mg., but the general adequacy of the diet is indicated by the fact that the animals grew to maturity, reproduced normally, and weaned their young in the usual time. The Mg. deficient rats grow quite as rapidly as those kept on the full diet; nevertheless, like the others, they manifest a lower resistance to infection.

*Casein, 15%
Starch, 54.4%
Butter fat, 8.0%
Lactose, 13.0%
Pure salt mixture, 9.6%
Vitamine supplement (lemon juice, tomato juice, yeast and cod liver oil).

Salt mixture, per 1000 cc. H_2O

Na_2CO_3	14.0 gm.	Citric acid	10.0 gm.
Ca CO_3	14.0 gm.	$FeCl_3$	0.4 gm.
Mg Co_3	4.0 gm.	HCl	11.3 gm.
K_2 CO_3	14.0 gm.	H_3PO_4	17.0 gm.
		H_2SO_4	1.8 gm.

Parallel with the determination of the effect of salt deficient diets on resistance, it seemed important to ascertain the physiological effect of the elimination of various cations from the diet. The observations thus far have been limited to the effect on the basal metabolism. The animals received the same diet as the trypanosome infected rats and were kept in the same cages and under the same conditions. Before each test the animals were starved for 20 hours, and then placed in a slightly modified Benedict apparatus. The results are given in Table II, the data being the averages of many tests.

TABLE II.

Diet	O consumption per kilo, per hour.	Res. Quo.
Standard	1335	0.77
Na poor	1550	0.725
Ca "	1540	0.77
K. "	1271	0.75
Mg. "	1170	0.77

The results indicate two things. First, that Ca and Na deficiency effect a higher oxygen consumption, while the K and Mg deficiency produce a reverse effect. This may account for the differences in the rate of growth. Second, while there is a definite change in the character of the respiratory quotient in the Na deficient rat, no such change occurs in the Ca., K or Mg deficient animals. Na deficiency apparently leads to a higher protein metabolism.

There is no apparent correlation between the disturbance in the quantitative metabolism and resistance, but there seems to be a definite relationship between the qualitative change and resistance. Na deficient animals have the same oxygen consumption as Ca deficient animals, and, a higher oxygen consumption rate than Mg deficient rats, but manifest a lower resistance than either of these groups. The Na deficient animals differ from the Ca and Mg and standard groups in the qualitative change of metabolism as indicated by the respiratory quotient, and this is correlated with lower resistance.

This line of investigation opens many possibilities. The cations evidently have a profound effect on metabolism and host resistance to infection. The experiments thus far were purposely radical in character in order to indicate the direction and nature of the changes effected. It remains to determine the effect of the partial elimination of the various cations from the diet on basal metabolism, specific dynamic action and resistance to various types of infections and toxins.

[1] Webster and Pritchet, *J. Exp. Med.*, 1924, xl, 397.

3865

Lactation Studies at Different Planes of Protein Intake.*

L. A. MAYNARD AND R. C. BENDER.

From the Laboratory of Animal Nutrition, Cornell University.

This is a preliminary report of part of an extensive study of the comparative effects of different planes of protein intake upon female rats with respect to the production of young, lactation, and the resulting condition of both mothers and offspring. Table I shows the rations employed in the present experiments, the dietary adjustments being such as to keep the yield of energy and the content of calcium and phosphorus constant while the protein in one mixture was increased through substitution for part of the carbohydrate in the other.

TABLE I. Rations Used.

Ingredients	Amounts	
	18% protein	50% protein
	gm.	gm.
Ground wheat	25.	25.
Dried skim milk	20.	20.
Dried yeast	3.	3.
Cod liver oil	3.	3.
Starch	38.	0.
Casein	8.	48.
NaCl	0.5	0.5
$Ca(H_2PO_4)_2 .2H_2O$	2.	0.
$CaCO_3$	0.5	0.5

TABLE II. Reproduction and Lactation Records.

Ration	No. of mothers		Lactations						Averages for 6 lactations
			1st	2nd	3rd	4th	5th	6th	
50% protein	4	Av. No. in litter	8.	8.	8.	5.8	5.8	8.	7.1
		Av. birth wt. (gm.)	5.2	5.4	5.1	5.4	5.2	5.3	5.3
		% reared	97.	71.	82.	50.0	86.0	95.0	80.2
		Av. wt. 20 days (gm.)	31.6	26.0	28.5	28.3	28.8	29.2	28.7
18% protein	4	Av. No. in litter	7.8	8.	7.6	8.	5.6	6.3	7.1
		Av. birth wt. (gm.)	5.2	5.3	5.4	5.3	4.9	5.8	5.3
		% reared	97.0	97.0	40.0	75.	94.	90.	82.2
		Av. wt. 20 days (gm.)	34.2	35.5	25.6	28.7	32.4	30.3	32.2

* These studies were begun by the senior author in the Laboratory of Physiological Chemistry, Yale University, under the direction of Dr. Lafayette B. Mendel.

Rats, under 100 days of age, weighing at least 160 gm. and previously fed alike on the stock ration, were placed on the 2 rations and mated. The male was removed when pregnancy was apparent. When the young were born, they were counted, weighed, and reduced to 6 (3 males and 3 females when possible). The mother and litter were weighed every 3 days until the 20th day, which was chosen as the period during which the weight of the young was a measure of the milk secretion of the mother. On the 22nd day the young were removed from the mother and the latter was remated for a second pregnancy and lactation. In this way each of the females was carried through at least 6 lactations, so that the possible cumulative effects of the diets could be noted.

Table II summarizes significant data for the first 6 lactation periods. The 2 rations have yielded identical averages for the birth weights and litter size. Though the figures for litter size and birth weights vary considerably from one lactation to another there is no certain trend either up or down with succeeding lactations. The records for the average time elapsing between successive lactations (not shown in the table) show no certain differences among the 3 groups. It may be concluded that a 50% protein ration is as effective for reproduction over a period comprising 6 lactations as is an 18% protein ration.

The records of the periodic weights of the mothers (not included in the table) show that in almost every lactation all the mothers maintained their own weights. This constitutes further evidence for the adequacy of the experimental rations over a series of lactations.

On examination of the kidneys of a few rats on both rations, all the kidneys from the high-protein group showed marked enlargements compared to those from the other groups, and also considerable degeneration. Large amounts of hyaline material were found distending the convoluting and collecting tubules, there was a multiplication of connective tissue with a decreased number of tubules, and coagulum was present in the lumen of the tubules. In contrast, the kidneys from the rats on the lower-protein ration showed little or no hyaline material and no connective tissue present, but did show some degeneration of the tubules. Though results thus far indicate increased kidney damage as a result of the high-protein ration, the recognized large margin of safety in kidney tissue is shown in the history of rat 902 on the high-protein ration. After the 10th lactation one kidney was removed from this rat. She recovered from the operation and remains in good health and condition 50 days after the operation and is now nursing another litter.

3866

Evidence of the Specificity of the Intracutaneous Pollen Test in Man.

ROBERT W. LAMSON AND GORDON ALLES.

From the Allergy Clinic of Dr. George Piness, Los Angeles, Calif.

Duplication of positive skin reactions to pollen extract was possible in most of the patients when 2 tests were made simultaneously, or when 2 or more weeks elapsed between the 2 tests. The pollens employed included a gramineae, a chenopodiaceae and 2 compositae. The majority of the individuals were positive to but a few pollens though some were positive to nearly all pollens.

Six species of *Artemisia* and 7 of *Atriplex* were tested on a series of patients. We have demonstrated that an individual may react to but 1 or 2 species of the 6 or 7 tested. Eighteen individuals reacted to but one of the 2 subspecies tested, while 6 patients reacted to the other subspecies.

In combination with the factor of accuracy previously discussed, these findings are considered evidence of the specificity of the intracutaneous test and, *a priori,* of the reacting substances in the pollen extract.

3867

A New Dietary Deficiency With Highly Purified Diets. III. The Beneficial Effect of Fat in the Diet.*

HERBERT M. EVANS AND GEORGE O. BURR.

From the Anatomical Laboratory, University of California, Berkeley.

Some years ago Osborne and Mendel[1] reviewed the work which had been done on low fat diets, and from an experiment with 6 young male rats concluded that "if true fats are essential for nutrition during growth, the minimum necessary must be exceedingly small." In view of the fact that their experimental animals were compared with the animals reared on a diet high in fat but low in protein, it seemed probable that both the controls and the experi-

* Aided by grants from the Committee for Research on Problems of Sex of the National Research Council and from the Bureau of Dairying of the United States Department of Agriculture. These funds have been generously augmented by the Board of Research and by the College of Agriculture of this institution.

mental groups were subnormal, thus leaving the question unsettled.†

It has been observed in this laboratory that our lard free diet 316 (Table I) is uniformly inferior to the high fat diet 232. Recently

TABLE I.
Composition of Diets.

Ingredients	Diet 232	Diet 316	Diet 519	Diet 520	Diet 522	Diet 525	Diet 526	Diet 527	Diet 528	Diet 529	Diet 533	Diet 534	Diet 535
Casein (commercial)	32	24.1		25.8		30.0	30.0	30.0	30.0	30.0	27.0	25.0	29.5
Casein (pure)			24.0		30.0								
Salts 185	4	3.6	3.8	3.8	4.5	4.5	4.5	4.5	4.5	4.5	4.0	4.0	4.0
Cornstarch (cooked)	40	72.3											
Sucrose (commercial)				70.4		45.5	45.5	45.5	45.5	45.5	59.0	66.0	48.5
Sucrose (purified)			72.2		45.5								
Lard	22				20.0	20.0	20.0				10.0	5.0	
Butter fat								20.0					
Corn oil (Mazola)									20.0				
Coconut oil (refined)										20.0			
Stearic acid													
Fatty acid from lard													18.0
Cod liver oil (Patch)	2												

† We have recently shown that their diet designated by us as our Basal Ration (Casein 18, cornstarch 54, lard 15, butter fat 9, salts-185 4) does not permit normal growth and ovulation in female rats.

TABLE II.
Showing the superiority of the high fat diet 232 over the low fat diet 316.

Diet	No. of animals	Average age at rupture of vaginal membrane	Average age at first oestrus	Total No. of ovulation cycles up to 90 days of age	Average No. of cycles per animal	Average wt. at 90 days
		days	days			gm.
232 + 0.7 g. yeast daily	15	39	41	136	9.1	206
316 + 0.7 g. yeast + 2 drops cod liver oil daily	15	47	54	63	4.2	188

TABLE III.
The Effect of Fat in the Diet.

Diet	Animal No.	Age at rupture of vaginal membrane	Age at 1st oestrus	Ovulation cycles. Length in days.	No. of cycles to 90 days	Wt. at 90 days	Average wt. of group at 90 days
		days	days			gm.	gm.
519 +							
1.0 gm. ether extracted yeast	W4936	44	48	5, 8, 7, 6, 13, 8, 5.		132	
	W4942	45	45	5, 5, 5, 4, 6, 5, 5, 6, 5, 8	23	136	136
3 drops cod liver oil	W4939	42	42	10, 6, 2, 3, 8, 7		142	
522 +							
1.0 gm. ether extracted yeast	W4958	41	42	5, 5, 6, 5, 6, 5, 5, 6, 6, 6		189	
	BH4957	41	48	6, 7, 5, 5, 5, 7, 5, 7	28	182	182
3 drops cod liver oil	BH4956	43	43	4, 8, 5, 7, 6, 5, 5, 5, 5, 6		174	

a carefully controlled experiment has been conducted to compare diet 316 plus 2 drops of cod liver oil (Patch) daily with diet 232. The groups of 15 females were made up of littermate sisters. Each diet was supplemented by 0.7 gm. yeast (Fleischman) daily. Table II gives the average results. It will be seen that the performance on the 2 diets is very different, the animals on diet 316 being retarded in growth, sexual maturity and ovulation. In fact, the rate of ovulation is decreased to less than 50% of its normal value. (The growth, maturity and ovulation here recorded for animals on diet 232 is equal to the normal on natural foods.)

TABLE IV.
Comparing the value of various fats as a supplement to low fat diets.

Diet	Animal No.	Age at rupture of vaginal membrane	Age at 1st oestrus	Ovulation cycles	No. of cycles to 90 days	Wt. at 75 days	Wt. at 90 days	Average wt. of the group at 90 days
		days	days	days		gm.	gm.	gm.
520+	B6402	78	80	9		144	162	
0.7 g. yeast	W6409	71	72	7, 4, 6, 5		114	132	
3 drops cod	G6422	78	80	6		118	138	
liver oil	W6429	68	72	7, 5	14	120	138	144
	BH6436	76	—	— — — —		112	138	
	W6393	63	63	5, 6, 6, 4, 5, 5		140	156	
525+	BH6403	54	54	7, 4, 7, 5, 5, 5, 5		170	191	
0.7 g. yeast	B6424	44	44	5, 6, 7, 10, 9, 5		166	178	
3 drops cod	W6404	61	62	4, —	41	142	158	177
liver oil	W6431	53	56	4, 7, 5, 5, 5, 5, 5, 5		170	178	
	W6411	44	44	3, 6, 6, 6, 5, 5, 5, 6, 6		155	168	
	W6394	40	40	5, 5, 7, 6, 5, 5, 5, 5, 5, 5		166	190	
526+	W6401	56	56	6, 5, 9, 7, 6, 8		126	140	
0.7 g. yeast	W6405	55	68	6, 6, 6, 4, 5		170	190	
3 drops cod	W6412	36	36	8, 5, 4, 4, 5, 4, 4, 4, 4, 4, 4, 8, 4	31	150	160	168
liver oil	B6425	51	81	— — — —		155	168	
	W6363	57	69	8, 6, 7, 6		150	174	
	B6468	53	53	13, 16, 10		155	174	
527+	W6406	56	85	9		120	150	
0.7 g. yeast	B6413	48	49	14, 6, 7, 5, 6, 6		168	188	
3 drops cod	B6426	45	48	6, 16, 11, 6, 5		128	150	
liver oil	W6433	54	54	11, 8, 7, 5, 5, 6	25	140	160	170
	B6469	54	61	5, 11, 7		135	152	
	BH6395	48	54	13, 10, 11, 11		185	220	
528+	W6407	76	81	9, 7		113	140	
0.7 g. yeast	W6420	59	78	6, 13		126	140	
3 drops cod	W6427	52	58	8, 10, 4, 5, 5, 6	22	155	178	164
liver oil	B6434	43	43	4, 6, 5, 6, 5, 5, 5, 5, 5, 5		165	188	
	B6470	72	89	— — — —		136	170	
	BH6396	55	77	8, 8		160	170	
529+	W6408	76	—	— — — —		80	104	
0.7 g. yeast	W6421	95	—	— — — —		80	98	
3 drops cod	W6428	76	—	— — — —	1	86	100	106
liver oil	BH6435	76	77	— — — —		104	120	
	W6471	92	—	— — — —		78	82	
	BH6397	77	77	12		108	120	
520+	W6410	58	61	5, 4, 6, 5, 5, 5, 5		150	160	
0.7 g. yeast	B6423	51	55	8, 12, 5, 7, 4, 6		175	196	
3 drops cod	W6430	69	77	6, 10		150	160	
liver oil	BH6437	57	59	6, 5, 6, 6, 5, 5, 5	26	164	160	171
10 g. fresh let-	W6472	77	87	— — — —		135	140	
tuce daily	B6398	61	68	11, 7, 4, 6		186	210	

TABLE V.

A further study of the value of lard and butter as supplements to a low fat diet.

Diet	Animal No.	Age at opening	Age at 1st oestrus	Ovulation cycles	Total No. of cycles to 90 days	Wt. at 60 days	Wt. at 90 days	Average wt. of group at 90 days
		days	days	days		gm.	gm.	gm.
520+	W7185	46	47	14, 12, 16		120	140	
0.7 g. yeast	W7200	48	50	9, 27		126	122	
3 drops cod	W7218	55	55		9	149	150	133
liver oil	BH7190	53	53			130	130	
daily	B7176	41	44	11, 8, 48		130	132	
	B7193	59	62	32		120	124	
525+	W7184	45	45	4, 5, 10, 13, 4, 4, 4		149	168	
0.7 g. yeast	W7199	48	57	4, 5, 10, 4, 3, 6	44	160	180	179
3 drops cod	W7217	48	48	11, 5, 5, 5, 5, 4, 8, 7		164	198	
liver oil	GH7189	46	46	11, 5, 5, 4, 4, 4, 4, 4, 4		140	172	
daily	GH7180	40	46	4, 5, 5, 6, 4, 6, 4, 4, 6		160	202	
	B7192	50	50	6, 8, 7, 5		149	170	
526+	BH7188	45	45	5, 9, 14, 5, 10		142	170	
0.7 g. yeast	W7219	45	45	15, 9, 7, 5, 6, 6		180	196	
3 drops cod	G7225	46	46	12, 5, 5, 5, 4, 5, 4, 4		149	160	
liver oil	W7178	44	45	9, 6, 9, 5, 6, 4, 7	38	172	194	180
daily	BH7183	46	47	7, 12, 5, 4, 5, 6, 5		140	180	
	BH7196	50	50	10, 11, 12, 44		158	180	
533+	W7187	45	45	4, 9, 5, 7, 5, 5, 4, 7		152	172	
0.7 g. yeast	BH7202	43	43	8, 5, 7, 5, 6, 6, 5, 5		138	152	
3 drops cod	B7224	42	42	4, 7, 6, 4, 6, 5, 6, 5, 8		140	140	
liver oil	BH7177	39	39	5, 6, 5, 9, 5, 5, 13	46	160	176	161
daily	G7182	35	35	7, 5, 6, 6, 5, 5, 5, 5, 5, 5		142	158	
	BH7195	51	70	7, 5, 5, 5		158	170	
534+	W7186	47	50	8, 5, 6, 6		150	176	
0.7 g. yeast	W7201	39	43	8, 6, 13, 8, 8, 5, 5, 5		160	164	
3 drops cod	B7223	47	47	5, 5, 5, 4, 5, 4, 5, 4		140	164	
liver oil	BH7191	49	52	7, 9, 5, 5, 5, 5, 5	39	138	142	163
daily	GH7181	40	44	5, 5, 5, 5, 10, 5, 5, 6		148	165	
	B7194	52	56	5, 6, 5, 4, 5		142	168	

By the substitution of sucrose for starch and of pure casein for commercial casein, the fat content of the diet has been further reduced (starch has usually 0.5 to 0.6% non-extractable fat and commercial casein carries a variable amount of butter fat). While working with these purer and simpler diets (Diets 519, etc., Table I) it was found that by the addition of lard to the diet, ovulation was always somewhat improved and growth was markedly improved. (Table III.) In fact, the animals which received the high fat diet 522 may be considered about normal in weight and ovulation, while those on the fat free diet 519, are greatly stunted.

In pursuing this study further a comparison has been made of the effects of various fats on the performance of young female rats. All these experiments have been done with wire bottom cages except for the comparison of diets 316 and 232.

The results summarized in Table IV show that 4 fats, lard, butter, cocoanut oil, and corn oil, when added to the sugar-casein diet, all significantly improved growth and ovulation during the first 90 days of life. The improvement was practically identical with that secured by the addition of lettuce. It may be noted that lard is not inferior (in fact, is slightly superior) to the other fats employed. This led us to repeat the experiments with lard (Table V), employing 5%, 10%, and 20% levels of this fat in contrast with littermate sister controls on the fat free diet 520 and on butter (diet 526). The ovulation history was virtually identical in all these groups but the growth in the groups receiving the lower levels of lard (5% and 10%) was slightly inferior.

Shortly after their 90th day of life an attempt was made to breed all females in these groups and to study the lactation performance on these diets. In all cases, during lactation the yeast dosage was

TABLE VI.

Lactation performance of animals on sugar-casein diet supplemented with various fats.

Diet	No. of mothers	No. of young to be suckled	No. of young weaned	% weaned	Aver. birth weight in gm.	Aver. weaning weight in gm.	% increase weight of young
520+ 0.7 gm. yeast 3 drops cod liver oil	1	6	6	100.0	5.2	20.6	295
520+ 10 gm. lettuce 0.7 gm. yeast 3 drops cod liver oil	6	36	30	83.0	5.1	26.0	410
525+ 0.7 gm. yeast 3 drops cod liver oil	3	14	13	93.0	5.7	28.6	402
526+ 0.7 gm. yeast 3 drops cod liver oil	6	36	31	86.0	5.2	36.7	606
527+ 0.7 gm. yeast 3 drops cod liver oil	3	18	15	83.0	4.8	30.0	524
528+ 0.7 gm. yeast 3 drops cod liver oil	4	24	23	96.0	5.3	32.6	516

increased 3 times in order to give the sucklings adequate vitamine B. Table VI gives the essential results. One may note that on the lard diets the worst rather than the best performance was exhibited. The butter diet is in respect to lactation easily the best of the series, furnishing, in fact, normal weaning weight. We are not yet prepared to explain the favorable influence of butter.

It will be noted that the addition of stearic acid in all cases stunted the animals badly and prevented the attainment of sexual maturity.

It seemed desirable to see if the valuable substance in fats could be located exclusively in any one of the 3 well recognized fractions —the fatty acids, the glycerol, or the non-saponifiable matter. Lard was saponified in 20% alcoholic potassium hydroxide on the steam bath and the non-saponifiable matter was extracted, washed and recovered in the usual way. The fatty acids were precipitated by dilute hydrochloric acid and recovered. Pure glycerol was fed instead of attempting a recovery of that substance from the aqueous layer left from the fatty acid. The comparison of these 3 mater-

TABLE VII.

Comparing the value of three fractions of lard in supplementing low fat diets.

Diet	Animal No.	Age at rupture of vaginal membrane	Age at first oestrus	Ovulation cycles to 90 days	Number of cycles to 90 days	Wt. at 60 days	Wt. at 90 days	Average wt. of group at 90 days
		days	days			gm.	gm.	gm.
520+	W7301	57				124	136	
0.7 g. yeast	W7304	63				130	138	
3 drops cod	BH7283	36	48	8, 4		156	150	
liver oil	W7290	65			9	120	112	133
N. S. M. from	B7279	45	50			119	139	
1.2 g. lard	W7326	36	54			114	124	
daily								
535+	BH7278	45	51	5, 5, 4, 4, 5, 4, 4, 4, 4		160	192	
(fatty acid	W7289	56	56	4, 8, 4, 5, 6, 4, 5		141	157	
from lard)	BH7303	50	50	17, 5, 5		132	152	
3 drops cod	BH7282	45	46	8, 10, 5, 20	31	154	176	158
liver oil	W7299	44	44	8, 6, 5, 5, 7, 6, 5, 5		144	162	
daily	W7327	59	94			100	108	
520+	BH7277	40	40	6, 7, 6, 16		120	122	
0.7 g. yeast	W7288	54				126	130	
3 drops cod	BH7300	51	51	5, 4, 4, 5		132	142	
liver oil	BH7281	57	74	6, 8	17	140	132	128
5 drops gly-	W7298	43	43	27, 39		128	132	
cerol daily	W7328	46	46	7, 5, 11, 12, 7		108	108	

ials was made and the results summarized in Table VII. They show that the group receiving the fatty acids was significantly superior to those receiving the non-saponifiable matter and the glycerol, neither of which could be described as improved by the addition, and they clearly indicate that the favoring substance has not been seriously hurt by the process of saponification.

Conclusions. (1) The highly purified and almost fat free diets 519 and 520 which give subnormal growth and ovulation, except when supplemented with small amounts of beef liver, lettuce or lard, can also be adequately supplemented by other fats, in particular by cocoanut oil, corn oil or butter. (2) The favorable substance in fats—possibly a new vitamine (F)—unlike Vitamines A, D and E, is not concentrated in the non-saponifiable fraction. It can be recognized in the fatty acid portion after saponification.

[1] Osborne and Mendel, *J. Biol. Chem.*, 1920, xlv, 145.